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HLA: THE MAJOR HISTOCOMPATIBILITY COMPLEX OF MAN

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Survival depends on the immune system's ability to recognize a multitude of foreign substances (antigens) and to respond to them. Although this defense mechanism is basic to survival in a hostile world of microorganisms, this same defense system becomes a major obstacle when attempting to transplant tissues from one individual to another or when malfunction of immune recognition triggers autoaggressive reactions. The major histocompatibility complex (MHC) genes encode proteins that are essential to this immune recognition. Products of the MHC have been grouped into three classes on the basis of structure, genetic origin, and function: Class I, Class II, and Class III. The human Class I molecules include HLA-A, HLA-B, and HLA-C; Class II molecules include HLA-DR, HLA-DQ, and HLA-DP; Class III molecules include the MHC-linked complement components (C2, C4, and BF), 21-hydroxylase (21OH), and tumor necrosis factor (TNF) (Fig. 30–1).

In an infection, the invading microorganism infects cells or is engulfed by immune cells such as monocytes. Inside the cell, MHC Class I or Class II molecules act as receptors for fragments of these microorganisms (Fig. 30–2). Once antigenic fragments have bound to the MHC molecules and have been translocated to the cell surface, receptors on T lymphocytes interact with the antigen-MHC complex triggering both humoral and cellular immune responses. T cell–specific cell sur-

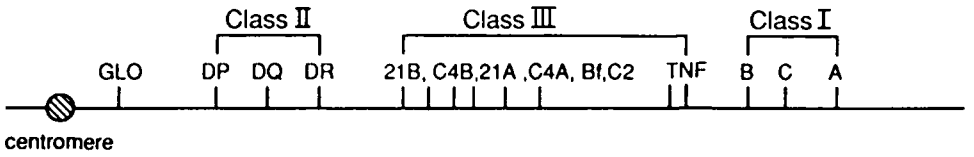


Figure 30–1. Map of the MHC gene complex on chromosome 6.

face molecules, CD8 and CD4, act to strengthen this cellular interaction and to transmit activation signals. Other cell adhesion molecules such as CD2 and its ligand, LFA-3, or LFA-1 and its ligand, ICAM-1, also act to increase the affinity of cellular interactions. The same pattern of interactions between a cell bearing an antigen-MHC complex and a T lymphocyte occur not only in the recognition of infecting microorganisms but also in the recognition of foreign histocompatibility molecules (alloantigens) found on an allograft and in the recognition of self antigens (autoantigens) during an autoimmune disease.

Also encoded in the MHC are components of the classic (C4, C2) and alternative (BF) complement pathways. These proteins are members of a complex group of interacting blood proteins whose primary functions are to produce inflammation, opsonize foreign material for phagocytosis, and mediate antibody-directed cytotoxicity against various cells and microorganisms (see Chap. 33). Other Class III genes encode for 21-hydroxylase, an enzyme of cortisol biosynthesis, and for tumor necrosis factor (TNF), a protein cytostatic or cytotoxic for tumor cells and a modulator of cellular growth and activity.

The impact of studies of the human MHC on clinical medicine was first felt in tissue transplantation, the area that gave the major histocompatibility complex its name. Because foreign MHC molecules are recognized as antigens by the graft recipient, it is beneficial to ensure that donor and recipient share HLA alleles. Since the MHC is extremely polymorphic, this is often a difficult task and requires close collaboration among the surgeon, the clinical HLA typing laboratory, and large organ-sharing networks. More recently, studies of the MHC have been important in understanding many autoimmune diseases that have immunologic imbalances or disturbances of immune regulation with an MHC-associated genetic component. Although the etiology of these diseases is not yet understood, intervention and therapy will depend on an understanding of immune recognition, the mechanisms of control of the normal immune response, and the relationship between particular MHC alleles and the propensity for the autoimmune disease.

The genetics, nomenclature, techniques of detection, structure, and function of Class I, Class II, and Class III products are discussed in this chapter. Also discussed are the importance of HLA matching for transplantation and the association of specific diseases with MHC gene products.

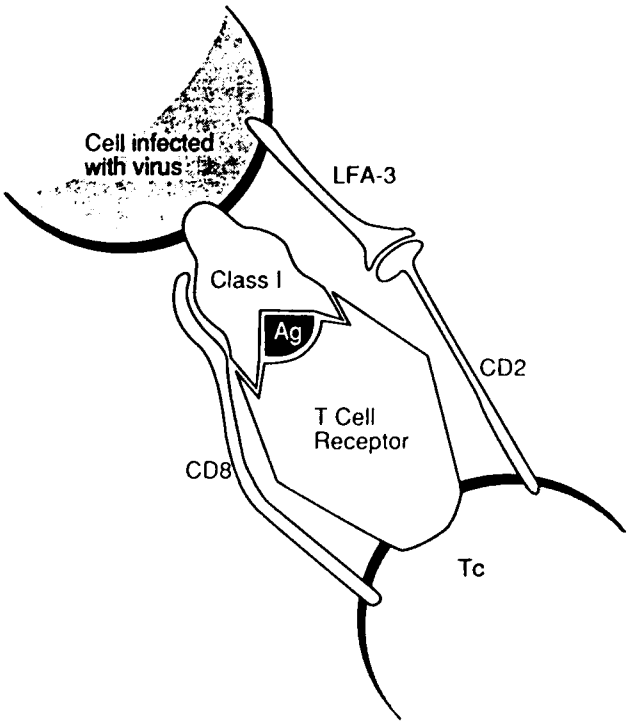


Figure 30–2. Model of the molecular interactions involved in antigen recognition.

GENETICS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Basic Genetics

Mendel's first law, the Law of Segregation, is based on the principle that hereditary characteristics are determined by factors that are distributed to progeny. In any one individual, these factors, or genes as they are now called, are present in pairs. During meiosis, the genes segregate randomly during formation of the gametes so that one of the pair, or haploid number, is transmitted by any given gamete. The double, or diploid number, is restored when the male and female gametes fuse to form the zygote. Thus, for a trait determined by one gene, there will always be four possible genetic combinations, each with equal probability of occurrence. These laws of segregation and random assortment apply to the genes of the MHC system. Definitions are given below for genetic terms that will be used in this chapter (Crow, 1976).

Gene The unit factor of inheritance.
Locus The position of a gene on a chromosome.
Allele An alternative form of a gene expressed at a single locus.
Homozygous Having identical or indistinguishable alleles at a locus.

Heterozygous Having different alleles at a locus.
Co-dominance The state in which each gene expresses its characteristic effect equally in the heterozygote.
Genotype The genetic constitution of an organism or individual.
Phenotype The observable characters produced by the genes.
Polymorphic Having two or more common distinct phenotypes maintained in a population.
Homologous chromosomes The two members of a chromosome pair that have corresponding gene loci, one derived from each parent.
Crossing over The exchange of segments between homologous chromosomes.
Recombination Reassortment of genes so that the gamete contains genes of both paternal and maternal origin. (When the genes are linked, this is accomplished by crossing over.)
Allo-(antigen, graft) Refers to antigenic differences between individuals of a single species.

Composition of the MHC

The major histocompatibility gene complex in man is located on the short arm of chromosome 6. It spans over 3500 kilobases, an area that contains enough genetic material to encode numerous proteins. The HLA (*H*uman *L*eukocyte *A*ntigen) genes of the MHC are encoded by at least six subregions: HLA-A, HLA-C, HLA-B, HLA-DR, HLA-DQ, and HLA-DP (see Fig. 30–1). Each subregion encodes a minimum of one cell surface glycoprotein. These HLA molecules, which are an integral part of the cell membrane, are highly polymorphic—that is, each of the HLA genes has multiple alternative forms (alleles) in the population. Indeed, they are the most polymorphic loci yet known in man. Such polymorphism is thought to be maintained because these molecules are used by the cells of the immune system to discriminate between self and non-self. Other molecules encoded within the MHC include three proteins of the complement cascade (C2, C4 (A and B), BF), 21-hydroxylase, and tumor necrosis factor (TNF).

Localization of MHC Genes

The genes of the MHC were assigned to chromosome 6 on the basis of cytogenetic studies of aberrant chromosomes. The map order of the MHC genes was determined by meiotic linkage analyses (studies of crossing over in families) and molecular biology techniques including gene cloning and pulsed field gel electrophoresis. The latter technique detects the presence of genes on single long fragments of DNA. The map order of genes within the MHC is HLA-A, C, B, TNF, C2, BF, C4A, 21OHA, C4B, 21OHB, DR, DQ, DP with HLA-A being distal to the centromere (see Fig. 30–1). The GLO-1 locus, which codes for an enzyme in erythrocytes that catalyzes the conversion of methylglyoxal and glutathione to S-lactyl-glutathione, marks the centromeric boundary of the MHC.

Inheritance

The MHC loci are closely linked—that is, they segregate en bloc to the offspring. The complex of linked genes that reside on one of the pair of homologous chromosomes and that segregate en bloc to the offspring is termed a "haplotype." Each individual inherits two MHC haplotypes—one from each parent and thus has two alleles for each of the loci. These genes are co-dominantly expressed. The inheritance of the MHC genes follows the rules of segregation set down by Mendel. Within a family, each child inherits one MHC haplotype from the mother and one from the father. By convention, the paternal haplotypes are generally designated a and b and the maternal haplotypes c and d. Thus, there are four possible MHC genotypes in the offspring: ac, ad, bc, and bd. Because the chances of inheriting a given genotype are random, the probability of occurrence of any one of the four genotypes is one in four. In a family with five children, at least two of the children will be HLA identical (assuming no crossing over). An example of a mating and its four possible genotypes is given in Figure 30–3. Although the MHC genes are closely linked, a number of families have been reported in which a cross-over has occurred (Fig. 30–4). The frequency of crossing over between two linked loci is controlled by the distance separating those loci and other characteristics of the intervening DNA that can increase or decrease the likelihood of recombination during meiosis.

Linkage Disequilibrium

The tendency for certain alleles at two linked loci to occur in the population significantly more frequently in the same haplotype than would be expected on the basis of chance alone is called linkage disequilibrium. The term is used in histocompatibility genetics somewhat differently than in general genetics where linkage disequilibrium refers to the higher than expected co-occurrence of two alleles. The expected frequency of two allelic forms at two loci to occur together is the product of the gene frequency of each allele in that population [$f_{expected} = f1 \times f2$]. The observed frequency is determined from family studies within the same population. Linkage disequilibrium is a hallmark of the human MHC and extends from HLA-A through HLA-DQ.

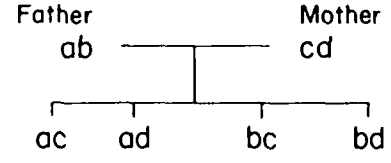


Figure 30–3. Segregation of alleles at a single locus.

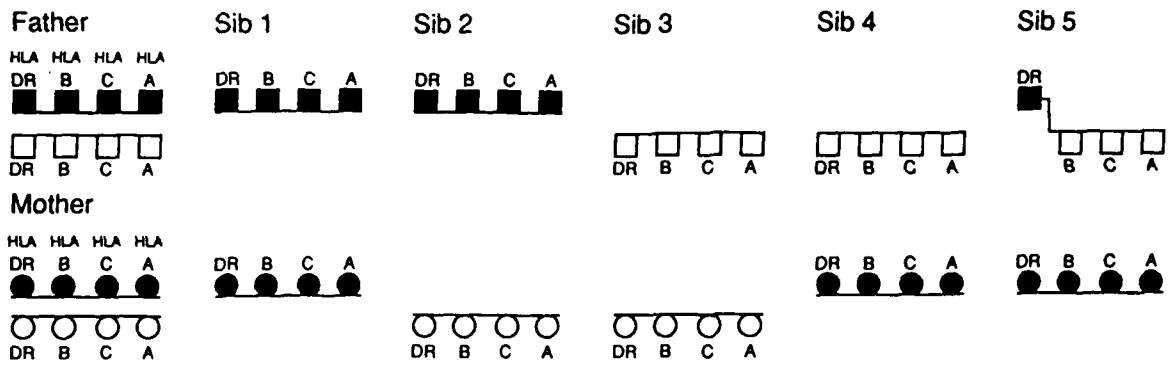


Figure 30–4. HLA haplotype segregation within a family. Sibling 5 is a recombinant between the paternal HLA-B and HLA-DR loci. This means that sibling 5 is HLA-A, HLA-B, and HLA-C identical to sibling 4, yet they are reactive to each other in MLC because of non-identity at the HLA-DR locus as a result of recombination.

The best known example of linkage disequilibrium is the A1, Cw7, B8, SC01, DRw17(3), DRw52a, DQw2a haplotype in Caucasians, which occurs approximately four times more frequently than would be expected by chance. DPw1 is also associated with this haplotype but with a lower frequency. This haplotype is found rarely in other ethnic populations. The significance of linkage disequilibrium as it applies to immune competence and disease is discussed in a later section of this chapter (see Extended Haplotypes).

Ethnic Variations

Characterization of HLA alleles of the human population groups of the world was undertaken systematically between June, 1970, and June, 1972, as the purpose of the 1972 International Histocompatibility Workshop (Dausset, 1973). The accumulated data from the 80 population groups investigated as well as from many subsequent studies (Aizawa, 1986) have provided several conclusions: (1) frequencies of HLA alleles differ significantly among ethnic subpopulations; (2) some HLA alleles are either confined to or found at a much greater frequency in one ethnic subpopulation compared with another; and (3) extended haplotypes and linkage disequilibrium differ among races. Therefore, for HLA and disease studies, both the ethnic background of the test group and the control group must be carefully evaluated and matched.

HLA-A, B, C SUBREGIONS—CLASS I MOLECULES

The HLA-A and B locus alleles were the first MHC alleles to be described. Anti-leukocyte antibodies in man were observed as early as the 1920s, but it was not until the 1950s that a systematic study began. Jean Dausset was the first (1952) to convincingly demonstrate anti-leukocyte antibodies (leukoagglutinins) in the blood of leukopenic patients and to suggest that these leukoagglutinins were probably alloantibodies because they did not react with leukocytes from the antibody producer but did react with a percentage of leukocytes from red cell group O unrelated individuals. Shortly thereafter, Rose Payne reported that sera from patients with febrile non-hemolytic blood transfusion reactions frequently contained leukoagglutinins that demonstrated allospecificity. In 1958, Dausset described the first HLA alloantigen MAC (now HLA-A2 + -A28) and showed it to be genetically determined. Originally, all the leukocyte specificities were thought to be the product of a single locus. A two-locus model, each with multiple alleles, was established by the identification of recombinational events that separated the two allelic series. These two allelic series were called the First or LA (now HLA-A) and the Second or Four series (now HLA-B). These names derived from the fact that the LA 1,2,3 allelic series was described by Payne in 1967 and the 4a,4b allelic series was described by van Rood in 1969. A third locus was first proposed in 1970; however, it was not confirmed by recombination until 1975. This third locus was designated HLA-C following the 1975 International Histocompatibility Workshop. Dausset was awarded the Nobel Prize for Medicine in 1980 for the original work on the human HLA system.

Structure of Class I Molecules

The Class I molecules, termed HLA-A, HLA-B, and HLA-C in the human, are heterodimers consisting of a transmembrane glycoprotein heavy chain (44 kD) noncovalently associated with β-2-microglobulin (12 kD) (Fig. 30–5) (Ploegh, 1981). The heavy chain of the Class I molecule spans the cell membrane and is oriented with its amino-terminus on the outside of the cell. β-2-microglobulin is associated with the extracellular region of the heavy chain and is necessary for cell surface expression. The extracellular region of the Class I heavy chain is divided into three domains designated α1, α2, and α3, each consisting of about 90 amino acid residues. The amino-terminal α1 domain contains a glycosylation site at the asparagine residue in position 86. The α2 and α3 domains each contain disulfide loops of 63 and 86 amino acids, respectively. The transmembrane segment of approximately 24 amino acids is mostly hydrophobic, whereas the intracellular carboxy-terminal segment of the molecule consists mainly of

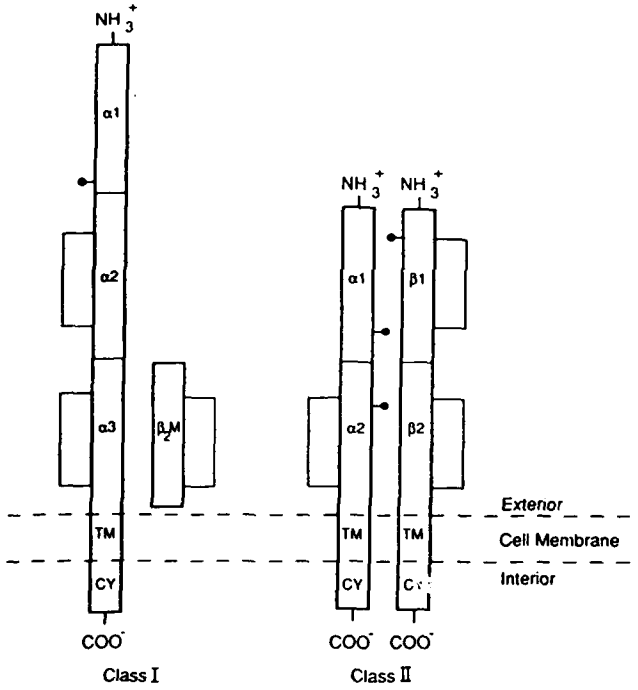
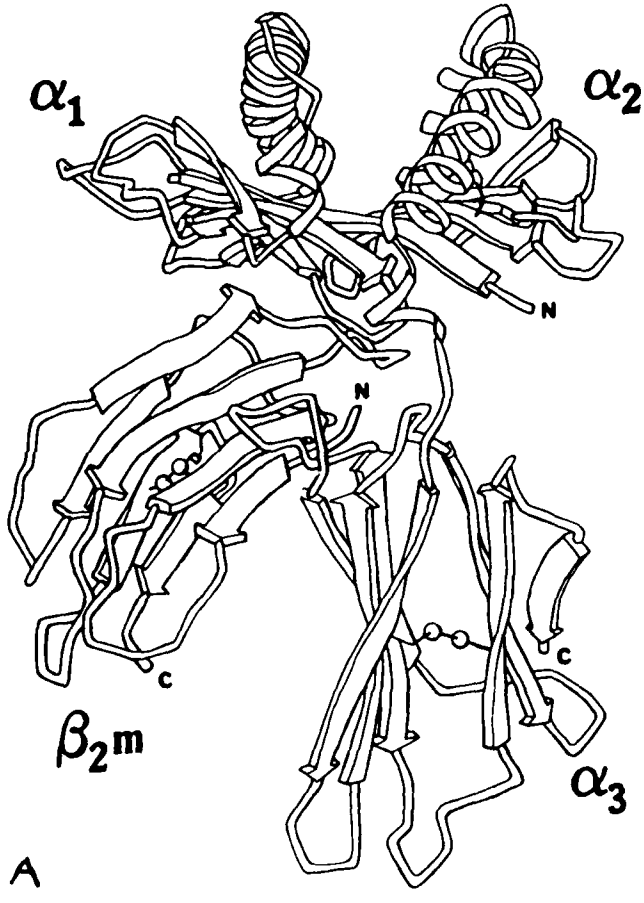


Figure 30–5. A schematic model of Class I and Class II molecules.

hydrophilic residues with a cluster of basic residues adjacent to the cytoplasmic surface of the cell membrane. Other membrane-bound molecules have been found to have similar clustering of basic residues that are thought to anchor the molecule in the membrane by charge interaction with the negatively charged membrane. The cytoplasmic portion of the molecule may be phosphorylated at certain serine residues that theoretically could transmit signals during an immune response.

Fresh insights into the way in which the HLA molecule interacts with antigen came when the Class I molecule was crystalized (Bjorkman, 1987a). The structure of the extracellular portion is shown in Figure 30–6. The molecule consists of two pairs of structurally similar domains: $\alpha 1$ has the same tertiary conformation as $\alpha 2$, and $\alpha 3$ and β-2-microglobulin also have similar tertiary conformations. The $\alpha 3$ and β-2-microglobulin domains are each composed of two antiparallel β-pleated sheets, one with four strands and one with three strands, connected by a disulfide bond. The $\alpha 3$ and β-2-microglobulin domains interact with one another through these β-pleated sheets and their structure closely resembles that described for the immunoglobulin constant region. The $\alpha 1$ and $\alpha 2$ domains are paired to form an eight-strand β-pleated sheet. This sheet is topped by two α-helices forming a groove or pocket at the top of the molecule (Fig. 30–6*B*). The sides and bottom of the groove are formed by side chains of the amino acids comprising the helices and β-pleated sheets. This groove is the likely site for the binding of antigenic subunits by the Class I molecule. It is postulated that amino acids in the β-pleated sheet at the bottom of the groove and in the helical regions play a role



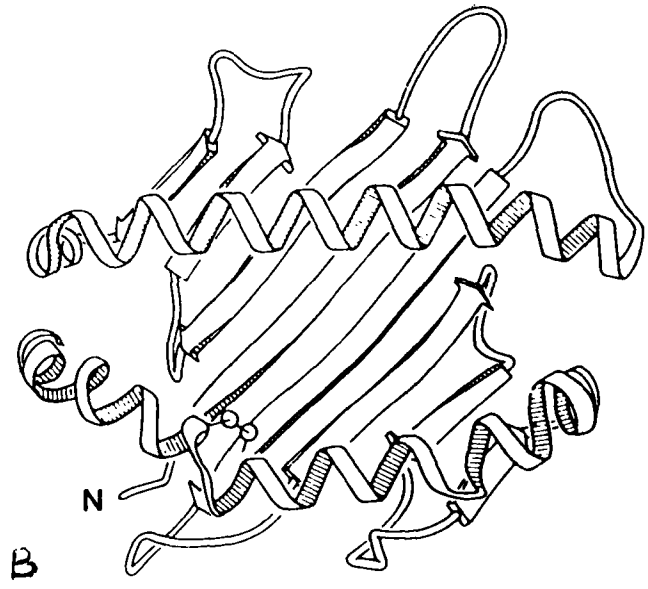


Figure 30–6. *A*, Three-dimensional model of the extracellular portion of a Class I molecule. (Reprinted by permission from Bjorkman, P.J., Saper, M.A., Samraoui, B., et al.: Structure of the human class I histocompatibility antigen, HLA-A2. Nature, *329*:506, 1987a. Copyright 1987, Macmillan Magazines Ltd.) *B*, Top view of the same molecule.

in binding of antigenic fragments, whereas other residues in the helical regions of the groove interact with the T cell during recognition of the Class I-antigenic fragment complex by a T lymphocyte (see Fig. 30–2).

Polymorphism of Class I molecules (Table 30–1) is defined by the use of (1) Class I specific antibodies (alloantisera and monoclonal antibodies); (2) cytotoxic T lymphocytes that recognize and kill in response to foreign or allogeneic Class I molecules *in vitro;* (3) isoelectric focusing of isolated Class I molecules; and (4) nucleotide sequencing of Class I genes. Most of the Class I polymorphism arises from amino acid sequence differences clustered in the $\alpha 1$ and $\alpha 2$ domains of the heavy chain (Parham, 1988). In the three-dimensional model of the Class I molecule, the variable residues form the antigen-binding groove (Bjorkman, 1987b). The variability in the amino acid sequence of these regions found in the Class I allelic products may affect the binding of particular antigenic fragments in the in-

Table 30–1. COMPLETE LISTING OF RECOGNIZED HLA SPECIFICITIES

A	B	C	D	DR	DQ	DP
A1	B5	Cw1	Dw1	DR1	DQw1	DPw1
A2	B7	Cw2	Dw2	DR2	DOw2	DPw2
A3	B8	Cw3	Dw3	DR3	DQw3	DPw3
A9	B12	Cw4	Dw4	DR4	DQw4	DPw4
A10	B13	Cw5	Dw5	DR5	DQw5 (w1)	DPw5
A11	B14	Cw6	Dw6	DRw6	DQw6 (w1)	DPw6
Aw19	B15	Cw7	Dw7	DR7	DQw7 (w3)	
A23 (9)	B16	Cw8	Dw8	DRw8	DQw8 (w3)	
A24 (9)	B17	Cw9 (w3)	Dw9	DR9	DQw9 (w3)	
A25 (10)	B18	Cw10 (w3)	Dw10	DRw10		
A26 (10)	B21	Cw11	Dw11 (w7)	DRw11 (5)		
A28	Bw22		Dw12	DRw12 (5)		
A29 (w19)	B27		Dw13	DRw13 (w6)		
A30 (w19)	B35		Dw14	DRw14 (w6)		
A31 (w19)	B37		Dw15	DRw15 (2)		
A32 (w19)	B38 (16)		Dw16	DRw16 (2)		
Aw33 (w19)	B39 (16)		Dw17 (w7)	DRw17 (3)		
Aw34 (10)	B40		Dw18 (w6)	DRw18 (3)		
Aw36	Bw41		Dw19 (w6)	DRw52		
Aw43	Bw42		Dw20	DRw53		
Aw66 (10)	B44 (12)		Dw21			
Aw68 (28)	B45 (12)		Dw22			
Aw69 (28)	Bw46		Dw23			
Aw74 (w19)	Bw47		Dw24			
	Bw48		Dw25			
	B49 (21)		Dw26			
	Bw50 (21)					
	B51 (5)					
	Bw52 (5)					
	Bw53					
	Bw54 (w22)					
	Bw55 (w22)					
	Bw56 (w22)					
	Bw57 (17)					
	Bw58 (17)					
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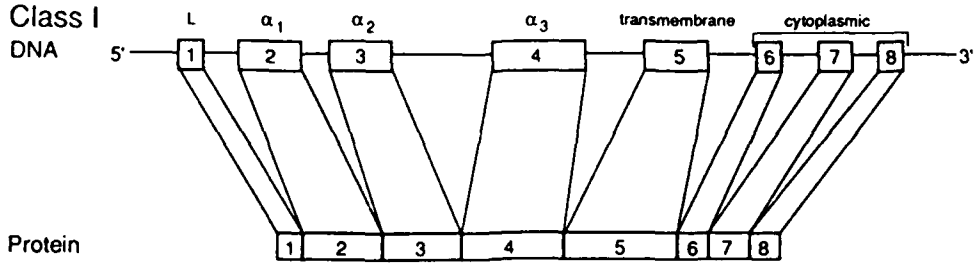

Figure 30–7. The exon and intron structure of a Class I gene and the corresponding protein. The leader peptide is not present in the mature protein.

itiation of the immune response. The $\alpha 3$ domain is highly conserved.

Class I Genes

The heavy chains are encoded within the HLA complex (see Fig. 30–1) (Jordan, 1985) and are highly polymorphic, whereas β-2-microglobulin is encoded on chromosome 15 and has not been found to be polymorphic in humans. Although three different Class I molecules (HLA-A/B/C) have been described serologically, as many as 20 additional Class I loci, such as HLA-E, have been identified by gene cloning. It is not yet clear if these additional genes are expressed as protein products.

The typical Class I gene is encoded by eight exons that correspond to the segments of the molecule just described (Fig. 30–7). The first exon encodes the 5′ untranslated region and a hydrophobic leader peptide. Exons 2 to 4 encode the three extracellular domains; exon 5 encodes the transmembrane region. The sixth and seventh exons encode the cytoplasmic region and the eighth exon encodes the 3′ untranslated region including the poly(A) addition site. The intervening sequences between the exons (termed introns) are transcribed into RNA but are removed during mRNA splicing.

Function of Class I Molecules

The Class I molecules are expressed on most nucleated cells of the body and also on platelets. The central function of the Class I molecules is the recognition and elimination of virally infected or tumor cells (Schwartz, 1985). For example, viral antigens are expressed on the cell surface in association with Class I antigens (see Fig. 30–2). CD8-positive cytotoxic T lymphocytes (CTL) recognize the viral antigen in conjunction with the Class I molecule. In the experimental system used to dissect this mechanism, cytotoxic T lymphocytes (CTL) were generated by *in vitro* stimulation with virally infected autologous cells. The recognition and lysis of target cells is doubly restricted to the priming virus strain and to certain HLA-A, B, and C allelic products present on the responder cell. Thus, only target cells sharing the appropriate HLA-A, B, or C antigens with the responder and infected with the appropriate virus strain will be lysed. This requirement for self MHC is termed "MHC restriction."

HLA-DR, DQ, DP SUBREGIONS—CLASS II MOLECULES

The Class II antigens in man were first recognized by their ability to stimulate allogeneic T cells in mixed lymphocyte culture (MLC). During the 1975 International Histocompatibility Workshop, the MLC was used to define the HLA-D allelic series (Thorsby, 1975). Because mixed lymphocyte cultures required seven days to perform, a rapid serologic detection of HLA-D was sought. In 1975, it was determined that alloantisera contained antibodies against molecules closely associated with the specificities previously identified as HLA-D with cellular techniques. Following the 1977 International Histocompability Workshop, these serologic specificities were termed DR for D-related specificities, because HLA-D and HLA-DR were observed to be associated (but not identical) to each other. Additional serologic reagents coupled with genetic and immunochemical techniques identified additional Class II molecules [MB1-3, MT1, DC-1 (now called DQw1-3), MT2 (now DRw52), and MT3 (now DRw53)]. Cellular techniques identified still another Class II molecule in the late 1970s. These molecules (now termed HLA-DP) were initially called secondary B cell antigens (SB) because they were usually weak or undetectable in a primary MLC and required a secondary phase stimulation in culture for detection. DP allelic products are still not adequately defined serologically.

Structure of Class II Molecules

The Class II HLA-DR/DQ/DP molecules are heterodimers consisting of two non-covalently associated transmembrane glycoproteins, an α-chain (33-35 kD) and a β-chain (26-28 kD) (see Fig. 30–5) (Kaufman, 1984; Brown, 1988). In contrast to the Class I molecules, both chains of the heterodimer are encoded within the MHC. Both polypeptide chains span the cell membrane and are oriented with their amino-termini on the outside of the cell. The extracellular regions of the α- and β-polypeptides are divided into two domains, designated $\alpha 1$ and $\alpha 2$ and $\beta 1$ and $\beta 2$, each con-

sisting of approximately 90 amino acid residues. The α-chain has one intrachain disulfide bond located in the $\alpha 2$ domain, and the β-chain has two disulfide bonds linearly arranged in the $\beta 1$ and $\beta 2$ domains, each spanning about 60 amino acids. The α-chain has two carbohydrate moieties, one high-mannose and one complex-type glycan, at amino acids 78 and 118. β-Chains have one complex-type oligosaccharide at amino acid 19. The membrane proximal $\alpha 2$ and $\beta 2$ domains of α- and β-chains are highly conserved and are homologous to immunoglobulin constant regions. A region of approximately 12 amino acids in length connects the second extracellular domain to the hydrophobic transmembrane region (23 amino acids) and a small intracytoplasmic domain (8 to 15 amino acids). From similarities in primary structure, it is thought that Class II molecules form a similar three-dimensional structure as the Class I molecules (Fig. 30–6) (Brown, 1988). During biosynthesis, Class II molecules are associated intracellularly with a third non-polymorphic glycoprotein known as the invariant chain and a sulfated proteoglycan molecule. The function of this biosynthetic intermediate is not yet clear.

Like the Class I molecules, the Class II molecules are highly polymorphic (Table 30–1). This polymorphism is defined by (1) Class II specific antibodies (alloantisera and monoclonal antibodies); (2) alloproliferative T cells that recognize and proliferate in response to foreign Class II molecules *in vitro;* (3) isoelectric focusing and two-dimensional gel electrophoresis of isolated Class II molecules; (4) hybridization of restriction endonuclease digests of DNA encoding Class II genes using locus-specific probes (a technique that detects restriction fragment length polymorphism (RFLP)); (5) hybridization of Class II genes with allele-specific oligonucleotide probes; and (6) nucleotide sequencing of Class II genes.

Class II Genes

The α- and β-chains of the Class II molecules are encoded within 1100 kilobases of the MHC complex (Fig. 30–8) (Korman, 1985; Bell, 1985). The region includes three subregions—DR, DQ, and DP—each of which encodes at least one expressed α- and β-gene. Other genes in the region are either pseudogenes or their expressed products have not been detected. Sequence comparisons suggest a successive series of gene duplications during the evolution of this gene complex. The

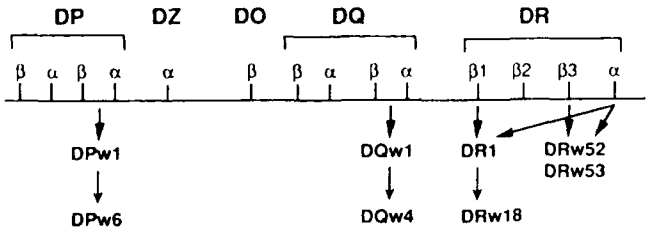

Figure 30–8. Map of the Class II region of the human MHC. Gene products encoded by each subregion are listed.

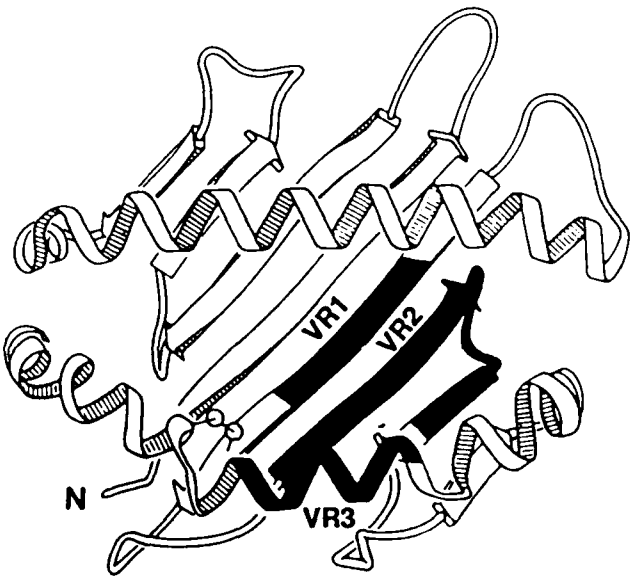

Figure 30–9. Three-dimensional model of the top view of the HLA-DR molecule with the variable regions indicated.

original split most likely gave rise to primordial α- and β-genes. More recent duplications generated the DR, DQ, and DP subregions followed by duplication to yield the α-/β-pairs within the subregions.

A typical α-gene contains five exons encoding: (1) 5′ untranslated region and signal sequence and the first few amino acids of the mature polypeptide; (2) $\alpha 1$ domain; (3) $\alpha 2$ domain; (4) connecting peptide, transmembrane region, cytoplasmic tail, and a portion of the 3′ untranslated region; and (5) the remainder of the 3′ untranslated region including the poly(A) addition signal. A typical β-chain gene is similar to the α-chain gene but has an extra exon for the cytoplasmic tail.

DR Subregion. The DR subregion encodes either one or two DR molecules depending on the haplotype. Some haplotypes such as DR1 and DRw8 express a single DR β-gene, whereas most other DR haplotypes express two DR β-gene products. The subregion contains a single expressed DR α-chain gene that is almost identical in different haplotypes differing for a single conservative amino acid substitution found in the cytoplasmic domain.

The most centromeric DR β-gene, DRB1 (Fig. 30–8), encodes a highly polymorphic β-chain that, when associated with the α-chain, exhibits serologic specificities DR1-DRw18 (Table 30–1). This molecule is usually the predominant Class II molecule on the cell surface, accounting for approximately 65 per cent of the total cell surface Class II molecules. There are multiple nucleic acid and, hence, amino acid sequence differences between DRB1 allelic products; however, the differences are located primarily in the amino-terminal $\beta 1$ domain and are clustered in three variable regions encompassing amino acids 9 to 13, 26 to 37, and 67 to 74 (Fig. 30–9).

The DR β-gene located adjacent to the DR α-gene (Fig. 30- 8) is expressed by some Class II haplotypes and is also polymorphic. This molecule represents approx-

Table 30–2. ASSOCIATIONS BETWEEN DR SEROLOGIC SPECIFICITIES

DRw52	DRw53
DRw17(3)	DR4
DRw18(3)	DR7
DRw11(5)	DR9
DRw12(5)	
DRw8	
DRw13(w6)	
DRw14(w6)	

imately 15 per cent of the cell surface Class II molecules. In cells expressing DR4, 7, and 9, this second DR molecule is termed DRw53 and is invariant in protein sequence among these haplotypes (Table 30–2). In cells expressing DR3, 5, and w6, this second molecule is termed DRw52. The DRw52 products expressed by different DR haplotypes may differ slightly in the amino acid sequence of the β-chain. Currently, there are three subtypes, DRw52a, DRw52b, and DRw52c. In cells expressing a DR2 haplotype, this second DR molecule also exhibits DR2 serologic determinants.

DQ Subregion. Two α- and two β-genes are encoded in the DQ subregion (Fig. 30–8). One set of α- and β-genes, DQA1 and DQB1, encode the DQ heterodimer that carries the DQw1-9 serologic specificities (Table 30–1). DQ molecules represent approximately 15 per cent of the Class II molecules on the cell surface. The differences among DQA1 allelic products are scattered throughout the first domain with a focus of variability at the region encoding amino acids 45 to 56. The differences between DQB1 allelic products, like DRB1, are clustered. Because both DQA1 and DQB1 are polymorphic and can associate in *trans* as well as in *cis*, heterozygotes can potentially express four different DQ molecules on their cell surfaces (Fig. 30–10). DQ molecules that carry the same DQ serologic specificity are found associated with several DR haplotypes (Table 30–3). Although these molecules carry the same serologic specificity, they may be composed of structurally different α- and β-chains. For example, DQw6 molecules encoded by the DRw15(2) and the DRw13(w6) haplotypes differ in amino acid sequence. The second set of α- and β-genes in the DQ subregion, DQA2 and DQB2 (sometimes called DX), are very similar to the DQA1 and DQB1 genes in structure; however, it is not clear if these genes express a functional protein product.

DP Subregion. The DP subregion also encodes two sets of α- and β-genes (Fig. 30–8). One set encodes the DP protein product, the other set is made up of pseudogenes. Currently, only six HLA-DP specificities are accepted by the international HLA typing community; however, DNA sequencing analysis has identified at least 14 alleles of the DP system (see Table 30–1). The polymorphism of DPA1 is limited to only a few allelic forms. The DPB1 chain is more polymorphic with the variability clustered in five regions encoding amino acids 8 to 11, 35 to 36, 55 to 57, 65 to 69, and 84 to 88 (Bugawan, 1988). Alleles of DPB1 share from none

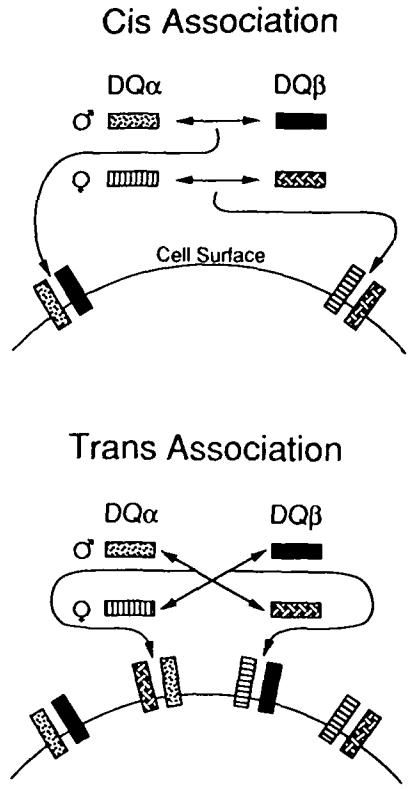

Figure 30–10. Model of the cis and trans associations of DQ alpha and beta chains.

(DPw1 versus DPw2) to four (DPw3 versus DPw6) of the sequences in these variable regions. Because of these shared amino acid sequences, monospecific alloantisera defining DP alleles are rarely found. The level of DP expression on the cell surface is very low (5 per cent of total Class II molecules expressed) and these molecules are usually detected by primed lymphocyte typing reagents.

Other Class II Genes

At least two other Class II genes have been described, DZ (or DN)-α and DO-β (see Fig. 30–8). It is not known if these genes are expressed as protein products, although it is likely that if an α-chain and a β-chain are encoded by these two loci, they would not form an α-/β-pair.

Table 30–3. COMMON DR/DQ ASSOCIATIONS

DQw1	DQw2	DQw3	DQw4
DR1	DRw17(3)	DR4	DRw18(3)
DRw15(2)	DR7	DRw11(5)	DR4
DRw16(2)	DR9	DRw12(5)	DRw8
DRw13(w6)		DR7	
DRw14(w6)		DRw8	
DRw11(5)		DRw13(w6)	
DRw8			

Linkage Disequilibrium in the Class II Region

Certain alleles of DR and DQ are inherited together more frequently than expected. A list of common associations is presented in Table 30–3. The frequency of some of these combinations [e.g., DRw11(5),DQw1; DRw9,DQw9(w3)] differs strikingly between populations of African versus Eurasian descent. Crossing over between the DP loci and the DR/DQ loci is common, resulting in a much looser association between alleles of DR/DQ and DP except for an association of DPw1 with DRw17(3).

Regulation of Class II Gene Expression

Class II genes are coordinately expressed in lymphoid cells and are inducible by γ-interferon in most cell types. Class II molecules are also modulated by other cytokines such as interleukin-4, tumor necrosis factor, and prostaglandins released by T cells during recognition of the MHC-antigen complex (see Chaps. 31 and 32). Two or more highly conserved DNA sequences are found in all Class II genes in a region upstream of the coding sequences. These upstream sequences bind proteins that regulate the expression of Class II genes. Mutations have been identified, some affecting these regulatory sequences, which simultaneously eliminate expression of all Class II genes, resulting in one form of severe combined immunodeficiency (SCID).

Function of Class II Molecules

Class II molecules are expressed on cells of the immune system including macrophages, dendritic cells, B lymphocytes, and activated T lymphocytes but not on resting T lymphocytes. Class II molecules act as antigen receptors in the immune response (see Fig. 30–2) (Schwartz, 1985). Specific binding between isolated Class II molecules and antigen or antigen fragments has been demonstrated by a number of laboratories. This provides a physical basis for MHC immune response genes.

Antigen receptors on CD4-positive T lymphocytes interact with the Class II–antigenic fragment complex triggering both humoral and cellular responses. In the experimental system used to dissect this mechanism, T lymphocytes were raised by *in vitro* stimulation with autologous cells that had been previously incubated with antigen. The recognition of the antigen-presenting cell is doubly restricted to the priming antigen and to particular HLA-DR, DQ, and DP allelic products present on the antigen-presenting cell. Each T cell activated by recognition of the complex of antigen and MHC can perform one or several functions. The T cell can help B lymphocytes differentiate to become antibody-producing plasma cells (see Chap. 31). It can help other T lymphocytes to differentiate into cytotoxic or suppressor cells. The T cell itself can become a cytotoxic cell, directly killing cells with the appropriate target (e.g., cells expressing Class II MHC molecules with the specific antigen), or a suppressor cell causing a reduction in the level of the immune response.

T cells can also produce a number of biologically important molecules (such as γ-interferon) that augment immune function and cause increased expression of MHC molecules on target cells. In addition, T cells produce growth factors such as interleukin 2, 3, 4, 5, and 6 and G-CSF, M-CSF, and GM-CSF. These growth factors can affect a wide range of cells from mature lymphocytes to hematopoietic stem cells.

The function of Class I and Class II molecules as antigen-binding receptors is similar; however, the types of antigen that bind to Class I and Class II molecules are thought to differ. Soluble and particulate antigens (exogenous antigens) are taken up by the cell in vesicles and associate with Class II molecules. In contrast, antigens synthesized *de novo* in the cell (endogenous antigens such as viral antigens) associate with Class I molecules.

COMPLEMENT REGIONS (C2, BF, C4A, C4B)—CLASS III MOLECULES

Although Class I and Class II molecules are distinguished by structural and functional similarities shared by the members of each class, Class III molecules can be defined only as "non-I and non-II" or "other," because their genes and their products have no common features (Alper, 1984). The proteins C2 and C4 of the classic pathway and factor B (BF) of the alternative complement pathway are encoded in a 120 kb region of genomic DNA about 300 kb from HLA-DR and 650 kb from HLA-B (see Fig. 30–1). C2 and factor B show considerable amino acid sequence homology and share a number of physiochemical and functional characteristics, suggesting that the two genes arose by tandem duplication of a factor B–like ancestral gene. Both are serine proteases (of the SERPIN family of plasma proteins) and mediate cleavage of C3 during activation of the respective pathways. C4, on the other hand, is related structurally and functionally to C3 and C5, and by virtue of containing a highly reactive internal thiolester, is related to C3 and the protease inhibitor α-2-macroglobulin. Although the gene for C3 is loosely linked to the MHC in the mouse, it is on an entirely different chromosome from the MHC in man. Human C4 is encoded by two highly homologous loci designated *C4A* and *C4B*. Immediately 3′ to each C4 locus is a locus for the adrenal steroid cytochrome P450 21-hydroxylase generally referred to as 21-hydroxylase or 21OH. In man, 21OHA is a pseudogene and only 21OHB is expressed. It has been found that the genes for tumor necrosis factor, TNF-α and -β, are located within the MHC as well and are situated 250 kb centromeric to HLA-B.

Factor B is synthesized by a gene termed *BF*. During activation of the alternative complement pathway, the protein is split into a 30 kD amino terminal Ba fragment and a 60 kD Bb fragment. In common with a number of C3-binding proteins, factor B (and C2) has

a variable number of 60 amino acid long homologous repeats. The protein exhibits moderate polymorphism with two very common alleles, *BF*F* and *BF*S*, two less common alleles, *BF*F1* and *BF*S1*, and a host of rare alleles. The amino acid substitution responsible for the common variants F and S are determined by residues found on the Ba fragment, whereas those for F1 and S1 are localized on the Bb fragment. On isoelectric focusing, BF F can be subdivided into two variants, BF Fa and BF Fb. DNA restriction fragment length polymorphisms have been found that either correspond to the F/S difference, the Fa/Fb difference, or further split BF F.

In contrast to the 6 kb *BF* gene, the gene for C2 is 18 kb, despite the fact that the mRNAs for the two proteins are closely similar at 2.6 and 2.9 kb. At the protein level, C2 shows minor polymorphism by isoelectric focusing. One allele, *C2*C*, accounts for over 95 per cent of C2 genes in most populations. In Caucasians, there is another allele, *C2*B*, with a frequency of around .02. A set of restriction fragment length polymorphism markers is detected in Sst I-digested genomic DNA of the C2 gene and are in strong linkage disequilibrium with the alleles in *BF* genomic DNA described earlier. C2 deficiency in Caucasians has an allele frequency of near .01.

C4 (as well as C3 and C5) is synthesized as a large single polypeptide of about 200 kD that undergoes post-synthetic processing involving proteolysis to a three-chain, disulfide bond–linked structure. The largest of these chains, about 87 kD, is the α-chain and carries the internal thiolester. During classic complement pathway activation, activated C1s cleaves a small peptide with anaphylatoxin properties from the amino-terminus of the C4 α-chain. This results in rupture of the internal thiolester, which is transiently capable of acylating nucleophilic groups in the immediate environment of the activated molecule. There are two distinct loci that encode for two forms of C4, *C4A* and *C4B*. Remarkably, C4A preferentially acylates amino groups, whereas C4B preferentially acylates hydroxyl groups. Thus, C4B is severalfold more hemolytically active, but C4A is more active at inhibiting the formation of and dissolving immune complexes than is C4B. In addition, C4A is usually more negatively charged than C4B, having a more slowly migrating α-chain in SDS gel electrophoresis. C4A usually carries Rodgers antigenic determinants, and C4B carries the Chido determinants. There is remarkably extensive genetic polymorphism in C4A and C4B proteins in all populations examined, detectable by agarose gel electrophoresis (Awdeh, 1980). In whites, there are four common structural alleles at the *C4A* locus, *C4A*2, 3, 4,* and *6*, and a common null allele, *C4A*Q0*, while *C4B* has a null allele, *C4B*Q0*, and three common alleles, *C4B*1, 2,* and *3*. Most striking also is the variation in C4 gene number on any individual haplotype. Although it is most common to have one expressed C4A and one expressed C4B, homoduplication and heteroduplication are common, as are haplotypes with only one expressed C4 gene. Inherited deficiencies of C2 and C4 will be dealt with subsequently.

Studies of the nucleotide sequence of the C4 genes have revealed that the differences between C4A and C4B protein sequences involve only about a dozen residues of the α-chain near the internal thiolester. This is the same region that carries the differences that determine individual variants and the presence of Chido or Rodgers determinants. Southern blot analysis with Taq I–digested genomic DNA and a 5′ C4 and a 21OH gene probe has revealed that about half of null alleles are the result of deletions. Moreover, the *C4A* deletion characteristic of the *C4A*Q0* on the haplotype with HLA-B8, DRw17(3) shows a characteristic restriction fragment with Taq I in this system. *C4A* genes are 22 kb in length, but *C4B* occurs in two forms, short (16 kb) and long (22 kb), all of which produce characteristic Taq I fragments. There are additional DNA polymorphisms that "split" protein polymorphisms and others that are characteristic of a single variant such as *C4A*6*.

Complotypes

The four complement genes in populations of haplotypes determined from family studies show significant linkage disequilibrium. That is to say, they occur in populations together as sets more frequently than expected from the frequencies of their individual alleles in much the same manner as alleles in the Rh and MNSs blood group systems. Furthermore, no crossovers have yet been documented between the complement genes. For these reasons, they are properly regarded as single genetic units, arbitrarily designated by their BF, C2, C4A, and C4B alleles. Thus, *BF*S, C2*C, C4A*Q0, C4B*1* is a complotype that in abbreviated form is SC01. There are more than a dozen complotypes in Caucasians that have frequencies of about .01 or higher (Table 30–4).

Extended Haplotypes

From the study of the distribution of complotypes in relation to HLA-B and HLA-DR specificities on nor-

Table 30–4. COMMON COMPLOTYPES*

Complotype	Frequency
SC31	0.430
SC01	0.127
FC31	0.096
SC30	0.053
SC42	0.040
SC61	0.034
FC30	0.031
FC01	0.029
SC02	0.029
SC21	0.022
SB42	0.019
SC33	0.014
SC2(1,2)	0.013
SC32	0.011

*Complotypes are given as abbreviated letters and numbers, four alleles in arbitrary order: BF, C2, C4A, and C4B.

Table 30–5. COMMON EXTENDED HAPLOTYPES

Haplotype	Frequency
B8,DR3,SC01	0.093
B7,DR2,SC31	0.059
B12,DR7,FC31	0.037
B17,DR7,SC61	0.034
B12,DR4,SC30	0.028
B14,DR1,SC2(1,2)	0.011
B40,DRw6,SC02	0.011
B15,DR4,SC33	0.009

mal Caucasian haplotypes determined in family studies, it became evident that there was striking linkage disequilibrium. One could easily recognize HLA-B, complotype, DR allele sets that showed statistically significant three-point linkage disequilibrium and that defined what we have called extended haplotypes. There are over a dozen common extended haplotypes in Caucasians; they are listed in Table 30–5. An analysis of HLA-A variation on these haplotypes revealed that this was limited and that each extended haplotype had a characteristic major HLA-A allele. It was also striking that most extended haplotypes and their major HLA-A alleles involved previously recognized HLA-A/B and HLA-B/DR pairs exhibiting significant linkage disequilibrium (Table 30–6).

The concept that has emerged is that these extended haplotypes, which account for at least 30 per cent of normal Caucasian haplotypes, have relatively fixed gross structure and DNA sequence and carry very similar, if not identical, alleles even when they are found in apparently unrelated individuals. All examples examined of [HLA-B44, FC31, DR4] carry an acidic variant of B44 and HLA-Cw5. [HLA-B8, SC01, DRw17(3)] and [HLA-B18, F1C30, DRw17(3)] carry different alleles of HLA-DQw2 and DRw52 and appear to differ by virtue of insertions and deletions in both the Class II and Class III regions. The two forms of DQw2 are differentiated by both RFLP and isoelectric focusing. Individual instances of each haplotype are identical to one another by all criteria tested.

Another important feature of extended haplotypes is their ethnic specificity. For the most part, they are highly characteristic of an ethnic subgroup and have lower frequencies or do not occur at all in other ethnic groups. They presumably had their origin in the group in which their frequency as intact haplotypes is highest.

From these considerations, we have developed the concept that extended haplotypes have fixity of DNA over at least the HLA-B/DR interval and, therefore, if they carry a disease susceptibility gene, then virtually all independent examples of that haplotype will carry that susceptibility gene. All of the alleles on the extended haplotype will show association with that disease. Conversely, if the extended haplotype does not have a disease susceptibility gene, then it and its constituent alleles will be "protective." If a susceptibility gene arose by mutation on an extended haplotype, this concept has to be somewhat modified to recognize disease-producing and non–disease-producing subsets of extended haplotypes. In any case, it is critical to know the ethnic distribution of extended haplotypes in order to evaluate whether the extended haplotype in patients is increased compared with truly ethnically matched control populations or is merely a marker for the ethnic distribution of the disease.

Although there are some superficial similarities between extended haplotypes and supratypes, as described by Dawkins and co-workers (1983) and both derived from the same phenomena, there are critical differences. Supratypes were defined as sets of HLA-A, B, DR, and complotype alleles in a phenotype, not necessarily on the same haplotype, and with no requirement that other alleles or intervening DNA be fixed or participate in the non-random association.

NOMENCLATURE

HLA terminology is designated by a World Health Organization (WHO) Committee for HLA Nomenclature based on results of international workshops in which typing reagents are exchanged between participating laboratories. The nomenclature originally adopted in 1975 was extensively modified in 1987. Following the Tenth International Histocompatibility Workshop, specific emphasis was placed on the HLA-D region nomenclature. [The following is paraphrased

Table 30–6. HAPLOTYPE PAIRS IN LINKAGE DISEQUILIBRIUM*

Haplotype	Δ/1000†	HF/1000‡	Haplotype	Δ/1000	HF/1000
A2,B12	27.2	64.5	B12,DR7	26.7	41.3
A1,B8	57.2	64.1	B8,DR3	62.3	70.1
A29,B12	27.3	33.1	B12,Dw2	18.4	30.3
A3,B7	18.5	28.3	B7,DR2	37.6	46.2
A1,B17	16.0	22.4	B17,DR7	22.8	29.4
A1,B5	13.7	20.6			
Aw23,B12	17.6	19.3	B12,Dw4	15.3	22.6
Aw30,B18	16.6	17.0	B18,DR3	12.9	18.2

*Data from Bodmer, W. F., and Bodmer, J. G.: Br. Med. Bull., *34*:309, 1978.
†Δ = observed haplotype (PQ) frequency − (frequency of P × frequency of Q).
‡HF = haplotype frequency.

Table 30–7. NEW NAMES FOR GENES IN THE HLA REGION

Name	Previous Equivalents	Molecular Characteristics
HLA-E	E, "6.2"	Associated with class I 6.2 kB Hind III fragment
HLA-DRA	DRα	DR α-chain
HLA-DRB1	DRβI,DR1B	DR β1 chain determining specificities DR1, DR3, DR4, DR5, etc.
HLA-DRB2	DRβII,DR2B	Pseudogene with DRβ-like sequences
HLA-DRB3	DRβIII,DR3B	DR β3 chain determining DRw52 and Dw24, Dw25, Dw26 specificities
HLA-DRB4	DRβIV,DR4B	DR β4 chain determining DRw53
HLA-DQA1	DQα1,DQ1A	DQ α-chain as expressed
HLA-DQB1	DQβ1,DQ1B	DQ β-chain as expressed
HLA-DQA2	DXα,DQ2A	DQ α-chain-related sequence, not known to be expressed
HLA-DQB2	DXβ,DQ2B	DQ β-chain-related sequence, not known to be expressed
HLA-DOB	DOβ	DO β-chain
HLA-DNA	DZα,DOα	DN α-chain
HLA-DPA1	DPα1,DP1A	DP α-chain as expressed
HLA-DPB1	DPβ1,DP1B	DP β-chain as expressed
HLA-DPA2	DPα2,DP2A	DP α-chain-related pseudogene
HLA-DPB2	DPβ2,DP2B	DP β-chain-related pseudogene

from "Nomenclature for Factors of the HLA System, 1987" (Human Immunology, *26*:3, 1989).] All genes in the HLA-D region are (1) prefixed by the letter D; (2) followed by a letter (for example, P) for the subregion (defined by the position and similarity of genes within a subregion); (3) followed by letters A or B indicating α- or β-chain gene (or related sequences in the case of pseudogenes); and (4) a number when there is more than one α- or β-chain gene (or pseudogene) in a subregion. The new names for genes in the HLA region are given in Table 30–7. For example, the α- and β-chains of the expressed DQ molecule are designated HLA-DQA1 and HLA-DQB1. New genes in the Class I region simply will be given single letter designations in sequence, omitting D, and prefixed by HLA. Provisional workshop assignments for specificities are designated by a "w" preceding the number. Following each International Histocompatibility Workshop, several old specificities are "split" as the definition becomes more refined until ultimately the reagents define a single allelic product. The most narrow definition of the specificity is called the subtypic specificity. The broader, shared specificities are called supertypic specificities. For example, B44 and B45 are subtypic specificities of the supertypic specificity B12. Thus, a cell that is either B44 or B45 positive must also be positive for B12.

Several different allelic products defined by nucleotide sequencing exhibit a single serologic or cellular specificity. Since the ultimate definition of an allele is the nucleotide sequence of a given gene, nomenclature was developed to name these molecularly defined alleles that are not yet defined by serologic or cellular reagents. Each allele defined by a nucleotide sequence will be given a unique number after an asterisk following the given gene name. The first two digits describe the most closely associated serologic specificity and the other two complete the allele number. This makes it possible to retain as far as possible the relationship between alleles and serologic specificities. Thus, the formal designations of the alleles corresponding to the HLA-A2 related variants defined by nucleotide sequence analysis are A*0201, A*0202, A*0203, and A*0204. The criteria for acceptance of a confirmed sequence includes depositing in publicly accessible repositories or data bases: (1) the cloned gene from which the sequence was derived; (2) the cell source(s) from which the cloned gene was derived; and (3) the nucleotide sequence of the gene or, if not available, the amino acid sequence of the allelic product. Table 30–1 shows the currently established HLA and workshop, "w," specificities. Currently, 24 A locus alleles, 50 B locus alleles, and 11 C locus alleles have been given WHO Nomenclature Committee assignments. Isoelectric focusing and nucleotide sequencing have identified additional alleles that have not yet been given a formal assignment. Alleles within the HLA-A and B loci are not numbered consecutively for historic reasons. Although some C locus specificities are considered well defined, the "w" designation has not been dropped because the notation would then be similar to that for the complement system. Notice that there is neither a 4 or 6 in either the A or the B allelic series. These numbers were reserved for the 4a,4b leukocyte system that was being investigated at the time the original nomenclature system was developed in 1967. Only in 1977 were experiments reported that showed that the 4a (now Bw4) and 4b (now Bw6) specificities reside on the same molecule as the B locus subtypic specificities, but at a different site. Thus Bw4 and Bw6 are a diallelic system, and all B locus molecules characteristically carry either w4 or w6 specificities.

There are 26 HLA-D specificities, 20 DR alleles, 9 DQ alleles, and 6 DP alleles. The HLA-D specificities are identified by mixed lymphocyte culture and "type" a cluster of Class II allelic products encoded by a haplotype. All of the HLA-D specificities retain the "w" designation because these specificities are only defined functionally and are probably a composite of DR, DQ, and DP (discussed under Mixed Lymphocyte Culture).

The nomenclature for genetic polymorphism of factor B, C2, and the duplicated loci for C4, C4A, and C4B, conforms with the International System for Human Gene Nomenclature. Null alleles and null variants are designated "Q0." Specific BF, C2, C4A, and C4B allelic sets form gametic units called complotypes. Complotypes are given in arbitrary order as BF, C2, C4A, C4B types; thus, SC01 represents *BF*S, C2*C, C4A*Q0, C4B*1.*

TECHNIQUES FOR DETECTING HLA ALLELIC PRODUCTS

Histocompatibility testing or HLA typing is performed in a limited number of laboratories because it uses specialized complex procedures and reagents. In addition, interpretation and translation of the results require considerable expertise. Histocompatibility laboratories are found in medical centers that have organ transplantation programs and/or as parts of large blood banks and transfusion services. Techniques are discussed in general in this section. For detailed procedures, the reader should refer to the American Society for Histocompatibility and Immunogenetics (ASHI) Laboratory Manual.

Serologic Detection of Class I and Class II Allelic Products

Originally, leukoagglutination was used; however, this technique is notorious for giving non-reproducible reactions. Lymphocytotoxicity testing, which was originally utilized in the mouse system by Gorer and Amos, was modified by Terasaki and McClelland for use in the human system. This assay represented a tremendous technical advance because reproducibility is greater than 98 per cent under controlled, standardized conditions.

Lymphocyte Preparation. Lymphocytes are employed routinely in HLA typing assays and are readily obtained from peripheral whole blood by layering onto a Ficoll-Hypaque gradient that separates blood cells according to density. Following centrifugation, one obtains a layer at the serum-Ficoll-Hypaque interface that is 99 per cent mononuclear cells. The separated peripheral blood lymphocytes (PBL) can be used for HLA-A/B/C typing.

To test for HLA-DR/DQ specificities, it is necessary to enrich for B lymphocytes or to use a special two-color fluorescent technique to simultaneously differentiate between B cells and T cells. Three enrichment procedures are currently employed by typing laboratories for the separation of the B lymphocyte subset: (1) Nylon wool adherence. B cells and monocytes preferentially adhere when a Ficoll-Hypaque–separated lymphocyte preparation is incubated on a nylon wool column. T lymphocytes are easily washed off the column because they are relatively non-adherent. Once the T cells have been washed off, the B cells can be removed by vigorous agitation while monocytes remain adhered. Although technique dependent, good B cell enrichment can be obtained. This technique is the one of choice of many clinical histocompatibility laboratories because it is inexpensive and does not require specialized equipment or reagents. (2) Magnetic beads. Magnetic beads coated with a monoclonal antibody specific for B lymphocytes (Dynal) can be used to positively select for B lymphocytes. Alternatively, magnetic beads coated with T cell–specific and monocyte-specific monoclonal antibodies can be used to remove non-B cells. Both procedures give >90 per cent B cells, although beads coated with anti-B cell monoclonal antibodies more consistently give a purer preparation. This procedure is relatively new but very promising. It is rapid, taking approximately 15 to 20 minutes, and requires small amounts (10 mL) of whole peripheral blood. (3) "Panning" (immunoadsorption of B cells to plastic dishes or flasks coated with an affinity-purified anti-human immunoglobulin fragment reagent raised against the $F(ab')_2$ fragment of IgG). Affinity-purified anti-Fab will bind to plastic by adherence. When a mononuclear cell suspension is placed in the coated flask, B lymphocytes bind to the antibody-coated surface by their immunoglobulin receptors. The unbound T lymphocytes are removed easily by washing. The immunogloblin positive cells (B cells) are recovered by competition using an elution medium that contains an excess of immunoglobulin. Although B cell purity is excellent and a uniform population is reproducibly obtained because of the positive selection, this technique is used primarily in specialized research laboratories. It requires special reagents and is more expensive and slightly more time consuming than the nylon adherence technique.

HLA Typing Sera. The major source of HLA typing antisera is from sera of multiparous women. During pregnancy, the woman mounts an immune response to the paternal antigens present on the fetus. Other sources of antisera are from transplant recipients, multi-transfused patients, and planned immunization of humans and of other primates. Sera from these sources are tested (screened) against a panel of lymphocytes of known HLA type. The panel is constructed to cover all HLA specificities with a minimum of two different cells when possible. Approximately 10 to 15 per cent of sera screened are useful for typing reagents. Thus, large screening programs must be maintained to identify reagents for one's own use and for exchange with colleagues if one produces local typing trays. Both bulk antisera and HLA typing trays are commercially available and are used by the majority of clinical laboratories. However, because the identification of new specificities and even allelic systems is through characterization of antisera with new and unexplained reactivity patterns, typing trays prepared with local and exchange sera are frequently used in research laboratories.

Because of the extreme antigenic complexity of HLA antigens, several antisera must be used to define each specificity. Most laboratories use trays from at least two different commercial companies. Frequently, from 140 to 210 antisera are used to define HLA-A/B/C specific-

ities and from 60 to 140 are used to define the HLA-DR/DQ specificities. Most antisera tend to have false positive and false negative reactions. Some rarer antigens are defined only by reactivity patterns of multispecific antisera because monospecific antisera are not available. Since many antisera are not truly monospecific and many exhibit some cross-reactivity (see later section), the reactivity of each antiserum should be thoroughly known by the individual interpreting the results. This requires stringent quality control of each new lot of typing trays with well-characterized reference cells.

Lymphocyte Microcytotoxicity Assay. HLA typing for the HLA-A/B/C/DR/DQ specificities is conventionally performed by a microlymphocytotoxicity assay. The HLA phenotype is determined by testing the unseparated lymphocyte preparation (PBL) or T lymphocytes (for HLA-A/B/C) or the enriched B lymphocytes (for DR/DQ) against a panel of well-characterized antisera. The assay is a two-stage test that uses only 1 μL of antiserum and 1 μL of a cell preparation containing 2000 lymphocytes. During the sensitization stage, the lymphocytes are incubated with the antisera generally for 30 minutes at 22° to 24°C. Carefully pre-screened and standardized rabbit serum as a source of complement is added in excess and the mixture is incubated for an additional 60 minutes. One modification of the technique employs a wash step, which removes complement inhibitory factors and increases the sensitivity of the assay before the addition of complement. Frequently, the incubation times are extended to 60/60 or 60/120 minutes, respectively, for B cell typing. If the lymphocytes carry a cell surface molecule recognized by complement-fixing antibodies in the serum, the antibodies bind to the cells and the cells are subsequently killed following addition of complement. Complement-mediated lysis results in changes in the permeability of the cell membrane that can be easily judged microscopically by the penetration of vital dyes. The assay is terminated by addition of eosin and formalin or trypan blue with EDTA. Reactions are read microscopically for per cent lysis and numerically graded. (8 = Strong positive, 85 or greater per cent lysis; 6 = positive, 60 to 84 per cent lysis; 4 = weak positive, 40 to 59 per cent lysis; 2 = questionably positive, 20 to 39 per cent lysis; 1 = negative, less than 20 per cent lysis.)

All lymphocytotoxicity procedures involve complement fixation, which can introduce a potential source of error and major variability in laboratory diagnosis. The source of complement is rabbit serum, but there are no standards on age, pool size, or potency. Therefore, meticulous quality control procedures by individual laboratories are of primary importance in the selection of rabbit serum for the lymphocytotoxicity assay. Each complement lot must be pre-tested for inherent cytotoxicity and for activity. The recommended method is to perform titrations of several HLA antisera and titrations of complement against cells from several individuals with appropriate positive and negative antisera. The same antisera and cells should always be used. In addition, a panel of lymphocytes should be HLA typed using the new lot of complement in parallel with the old lot of complement. Commercial typing trays provide their own complement that has been quality controlled with the typing trays. The complement provided should be used but still should be quality controlled to assure strength of activity.

Cross-reactivity. Many HLA antisera react with more than one allelic product. This phenomenon can result from cross-reactivity and/or from polyspecificity of the antisera and is responsible for the complex reactivity patterns of antisera. Polyspecific sera contain two or more HLA antibodies that can be absorbed easily to remove one antibody with little effect on the reactivity of the other(s). Polyspecific sera may contain Class I antibodies specific for any combination of allelic or non-allelic molecules (e.g., an antiserum may contain both HLA-A3 and HLA-B5 specific antibodies). Cross-reactive antisera contain, most frequently, a single antibody that reacts with an antigenic determinant shared among several different HLA alleles. Adsorption of cross-reactive sera for the homologous (e.g., the strongest) reactivity will also remove the reactivity for the other cross-reactive HLA alleles. Cross-reactions occur most frequently among allelic products encoded by the same locus but can occur between allelic products encoded by different loci (e.g., A2 + B17). Diagrams of cross-reactivity at the A locus and at the B locus are given in Figures 30–11 and 30–12. Cross-reactivity appears to result from shared amino acid sequences among alleles. Examples of the localization of cross-reactive determinants are given in Figure 30–13. Cross-reactivity between allelic products at the HLA-A and at the HLA-B loci has been extensively studied and has been used to group alleles into cross-reactive groups (CREG)

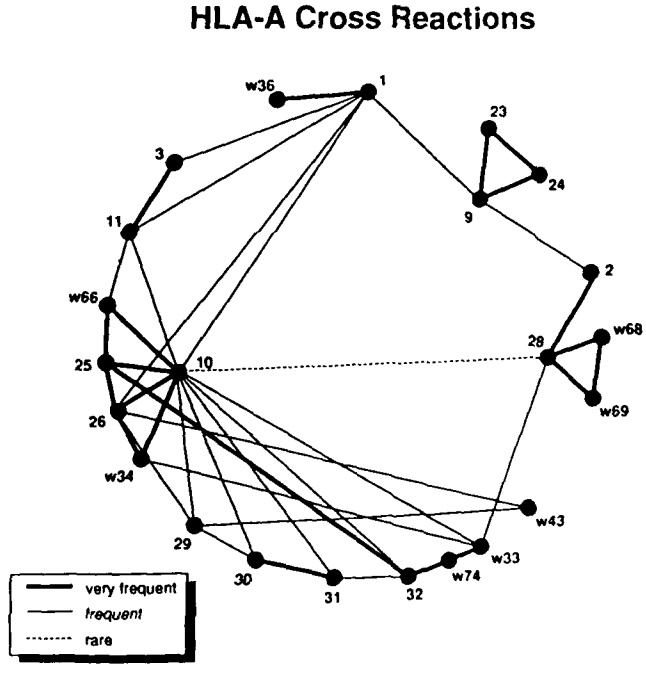

Figure 30–11. Cross-reactivity at the HLA-A locus. (Modified from that of Prof. W. Mayr, Vienna.)

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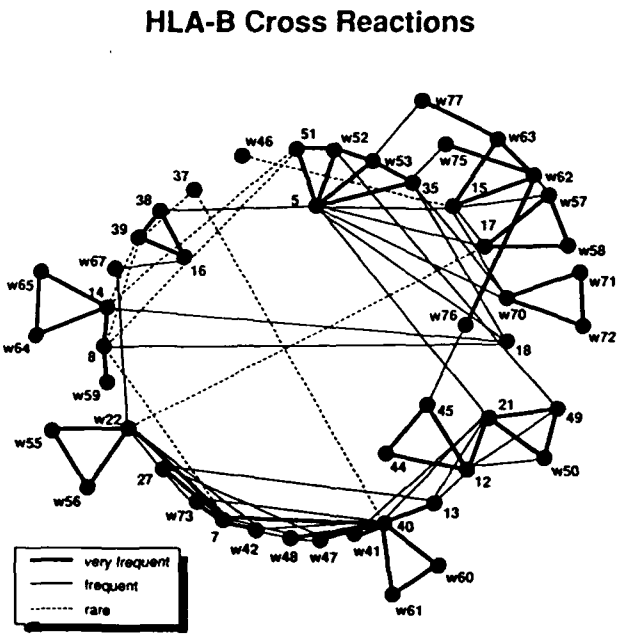

Figure 30–12. Cross-reactivity at the HLA-B locus. (Modified from that of Prof. W. Mayr, Vienna.)

(Rodey, 1987). Class I molecules within a CREG presumably share one or more determinants that are not shared by specificities in another CREG and are more closely related to each other than to other Class I molecules. An antigenic determinant shared between members of a CREG is called a public specificity. Examples of CREGs are: A2 CREG: A2, A28(w68 + w69), A9(w23 + w24); A1 CREG: A1, A11, A10(25 + 26), A3; B7 CREG: B7, B27, B22(w55 + w56), B42, B40(w60 + w61).

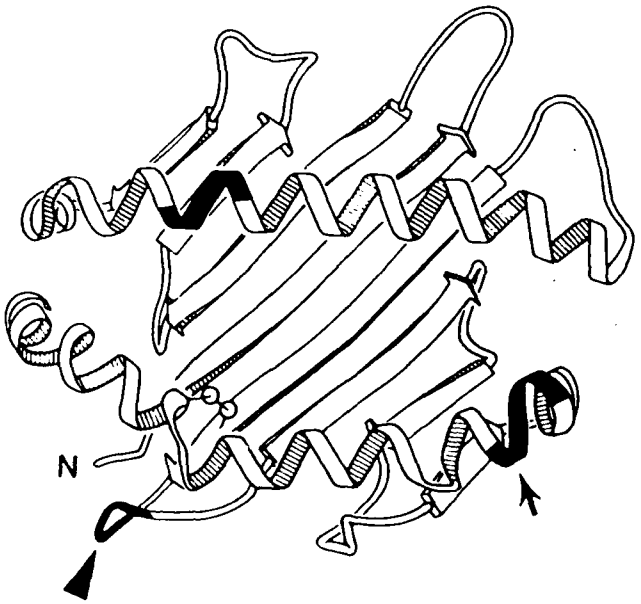

Figure 30–13. Top view of a Class I molecule with polymorphic amino acid residues indicated for cross-reactive specificities A2 + A28, A2 + Aw69, and A2 + B17. (From Parham, P., et al.: HLA-A, B, C. *In* Dupont, B.: Immunobiology of HLA. New York, Springer-Verlag, 1989, p. 17)

Cellular Detection of Class II Molecules

Peripheral blood T cells are able to recognize and respond to a wide variety of antigens by undergoing cell division *in vitro*. This response is measured by analyzing the level of DNA replication, using radioactive thymidine incorporated into the dividing cells. The molecules that stimulate this replication can be either antigens previously experienced by the individual (e.g., influenza antigens or tetanus toxoid) or alloantigens (e.g., HLA molecules). The response of one cell in tissue culture to the alloantigens on the surface of a second cell is called the mixed lymphocyte culture (MLC) or mixed lymphocyte reaction (MLR). The MLC is considered an *in vitro* measure of Class II disparity between individuals. The response is made unidirectional by preventing cells from one of the two individuals from replicating by treating those cells with radiation or mitomycin-C prior to addition to the culture.

Mixed Lymphocyte Culture. Early family studies by Bach and Amos (1967) indicated that cells from siblings who were serologically identical for HLA-A and HLA-B failed to respond to each other in MLC. This hypothesis was challenged by Yunis and Amos (1971), who reviewed evidence from intra-HLA recombinant families that suggested that HLA-A and -B were not the MLC-stimulating determinants but that stimulation was caused by molecules encoded by genes adjacent to HLA-A and -B. Further, Thorsby (1975) showed that cells from most unrelated individuals who typed identically for HLA-A and -B specificities responded to each other in MLC. These MLC-stimulating determinants became known collectively as HLA-D (see Table 30–1) and represent determinants found on HLA Class II molecules.

The original method to type for HLA-D was called the homozygous typing cell (HTC) method. This method is very difficult and expensive and has been supplanted primarily by other methods. HTCs are derived from individuals who are homozygous—that is, they have two identical copies of each Class II gene. In the early 1970s, it was hypothesized that if the irradiated HTC was used as a stimulator in an MLC, a heterozygous responder cell that shared one copy of each Class II gene with the HTC would not detect any foreign alloantigens on the stimulator cell and, thus, would not proliferate. This lack of response is termed a typing response. The HLA-D specificity defined in this way represents a summation response of a responder cell to differences in the multiple determinants on HLA Class II molecules (DR, DQ, and DP) encoded by the irradiated stimulator cell haplotypes. The relative importance of each amino acid sequence difference of each allele of each Class II molecule in generating an

MLC between different HLA Class II alleles is not clear; however, the response to DR molecules appears to predominate. The DQ molecule expressed by a haplotype also plays a role in the MLC, although considerably less than DR. The role DP plays in mixed lymphocyte stimulation is minor, because secondary or primed responses are usually needed to define these specificities; however, low level stimulation can be detected in an MLC where HLA-DP is the only difference between two otherwise identical siblings as observed in families where an HLA-DQ/HLA-DP recombination has occurred.

DR haplotypes vary in complexity as judged by HTC typing. For example, DR4 is subdivided into Dw4, Dw10, Dw13, Dw14, and Dw15 subtypes; DR2 is subdivided into Dw2, Dw12, Dw21, and Dw22 subtypes. Data on the biochemical basis of MLC-defined HLA-D specificities suggest that for DR2 and DR4 haplotypes, there are different, yet overlapping, sets of DR and DQ polypeptides associated with each HLA-D type. The structural basis of the differences between DQw3-associated DR4 molecules from different HLA-D specificities appears to be a small number of amino acid differences predominantly in the third variable region (amino acids 67 to 74) of the DRB1 encoded protein sequences. For example, the DRB1 sequence differences range from five amino acid differences between DR4,Dw4, and DR4,Dw10 to one amino acid difference at position 74 between DR4,Dw13, and DR4,Dw14. Thus, although the DR molecules share the DR4 serologic determinant, they are composed of different DR β-chains. These differences are detected by MLC.

Mixed Lymphocyte Culture (MLC) Techniques. The basic miniaturized MLC culture and the harvesting techniques used today were developed in 1969 by Hartzman. Peripheral blood mononuclear cells free of platelets and erythrocytes are obtained by using the Ficoll-Hypaque gradient technique of Boyum. This lymphocyte separation method is particularly important as it removes most granulocytes that can non-specifically suppress *in vitro* replication. Mononuclear stimulator cells are irradiated (generally 1500 rads) or treated with mitomycin-C (generally 25 to 50 μg/mL). Responder and stimulator cells are then added to 96-well tissue culture plates in a total volume of 50 to 200 μL of tissue culture media containing several supplements including serum and antibiotics. Cells are incubated at 37°C. in a humidified atmosphere for five to six days, at which time 1 to 2 μCi ^{3}H-thymidine (2 to 20 Ci/mM) are added to each well followed by 6 to 18 hours of additional incubation to permit uptake and incorporation of thymidine. In culture, cells undergo the process of recognition and then begin to divide. Under usual conditions, no thymidine incorporation can be detected for 48 to 72 hours, although increased glucose metabolism can be detected within 1 hour, and insulin and transferrin receptor changes can be identified within several hours after mixture. After the initial "latent" phase, replication begins and continues at a rapid pace up to the sixth to eighth day of culture, depending on the "strength" of the stimulus. Greater cellular activation results in earlier peak responses. During this time, many large blast lymphocytes can be seen in the culture, the number being proportional to the uptake of thymidine. Following culture, cells are washed free of unincorporated tritiated (^{3}H) thymidine onto small fiberglass filters using a semi-automated harvester. The amount of ^{3}H-thymidine remaining on the filter and, thus, the amount of thymidine incorporation into the cells is assayed with a scintillation counter.

T Cell Cloning. T cell clones are the cellular counterpart of monoclonal antibodies. Cloned T lymphocytes (TLC) are reagents that can define a single small difference in Class II molecules between the responder and the stimulator cells. Although the method is technically very demanding, a panel of cloned alloreactive T cells derived from a single *in vitro* priming of lymphocytes can be derived that will define the number and predominance of different Class II determinants (epitopes) present on stimulating lymphocytes. Most of these TLC recognize DR molecules, a smaller percentage recognize HLA-DQ or DP determinants. Allospecific TLC can replace cellular typing with homozygous typing cells. However, as a practical matter, typing with DNA probes will likely supersede other typing methods, leaving cellular methods as research tools to analyze the functional properties of the Class II molecules.

Primed Lymphocyte Lines. Extended culture of a mixed leukocyte reaction for at least two days after the peak of tritiated thymidine incorporation (usually 10 days total) allows the cells in culture to return to a resting state. The majority of these cells are the progeny of cells that have recognized the priming HLA Class II specificities and can be restimulated by the HLA antigens used for the original priming. This restimulation or secondary response is more rapid than the original MLC response, peaking at three days. Thus, primed cells can also be used to type for Class II specificities. Primed lymphocyte typing (PLT) reagents can be generated that detect alleles of DR, DR52/53, DQ, and DP. In fact, HLA-DP specificities were originally defined by PLT.

T Cell Priming and Cloning Techniques. After the stage of maximum division in the MLC, many of the replicating cells revert to small quiescent lymphocytes and no longer incorporate large amounts of thymidine. These resting cells remain stable in tissue culture, if adequate nutrients are provided, for another 5 to 30 days. The return to quiescence and stability of these stimulated cells is of central importance to a number of other tests of cellular recognition. During the initial priming, the number of cells capable of recognizing a given stimulating determinant has increased in the culture. As there are many stimulator cells with many antigenic determinants in any one "bulk" leukocyte culture, many clones of T cells reactive to each of the many antigenic determinants present on each Class II molecule proliferate. In addition, these primed cells are capable of rapid restimulation *in vitro* when used in subsequent tests.

To clone individual T lymphocytes, primed cells are restimulated in limiting dilution cultures so that large

numbers of cells can be derived from a single primed lymphocyte culture. Primed cells are diluted and distributed into microtest plates (10 to 20 μL/well) along with T cell growth factor (the spent culture medium obtained from stimulated lymphocytes that contains interleukin 2 [IL-2]) and the irradiated specific stimulator cell in high excess (10,000 cells per well). The stimulator cells also provide factors needed by the T cells for growth. After 5 to 10 days of incubation, replicating lymphocytes can be detected in some wells. These replicating cells are transferred to larger wells along with additional feeder cells and IL-2. A modest variation of this procedure allows one to generate clones of T lymphocytes directed against antigens such as viruses. These antigen-specific clones are developed by priming with the desired antigen followed by cloning in the presence of feeder cells that share Class II alleles with the responder cell. Once either allospecific clones or antigen-specific clones are generated, very large numbers of these cells (10^7 to 10^{10}) can be produced and cryopreserved for extended analysis.

DNA Typing of Class II Alleles

Although serologic and cellular typing of HLA-DR, -DQ, and -DP have been extremely useful, there are several drawbacks to these techniques. Both serologic and cellular reagents are difficult to obtain and characterize. In addition, the results, which are very sensitive to differences in closely related Class II allelic products, are often difficult to interpret because of cross-reactivity caused by shared determinants. With the advent of rapid methods for the isolation of Class II genes and the determination of the nucleotide sequences of Class II alleles, it has been possible to use hybridization of allele-specific oligonucleotide probes (ASOPs) to DNA to identify Class II alleles. The use of several different oligonucleotides to define a specific Class II allele may be required when no unique sequence can be identified. This is especially true for the typing of DP alleles, which often share variable region sequences. Oligonucleotides can be synthesized that contain short stretches (20 to 30 nucleotides) of DNA sequence unique to specific DR, DQ, and DP alleles. These unique sequences are usually found in the Class II β-chain variable regions. DNA is isolated from peripheral blood lymphocytes or any other source of cells. The sensitivity of detection is enhanced greatly by the amplification of DNA or RNA encoding Class II genes using a technique termed the polymerase chain reaction or PCR (Saiki, 1988). This amplified DNA is spotted onto a nylon filter and hybridized with a set of oligonucleotides capable of identifying each allele. Hybridization conditions are adjusted so that the oligonucleotides will anneal to denatured DNA containing the Class II allele from which the oligonucleotide sequence was derived. Figure 30–14 illustrates the approach using an oligonucleotide probe (dot blot) for the DQB1 allele, DQw8, to identify patients with insulin-dependent diabetes who carry this allele (Todd, 1987). At present, oligonucleotide typing is used primarily in research laboratories; however, modifications to adapt this technology to the clinical laboratory are being developed and will be used in the Eleventh International Histocompatibility Workshop.

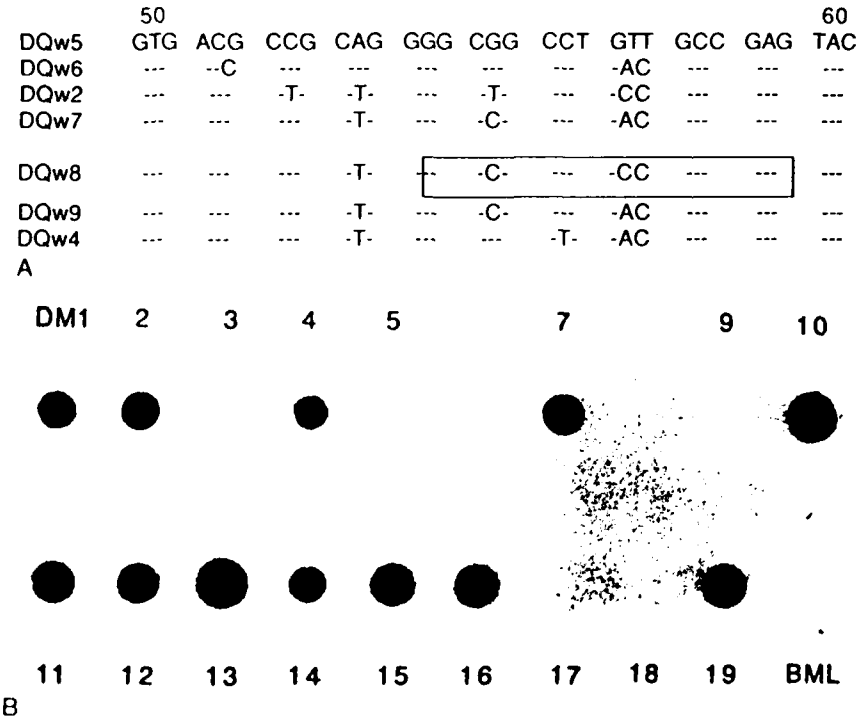

Figure 30–14. *A*, Nucleotide sequences of DQ β-alleles. The sequences presented cover the codons for amino acids 50 to 60. A dash indicates that the nucleotide is identical to the top sequence. An oligonucleotide that is specific for the DQw8 β-allele sequence is boxed. *B*, Oligonucleotide dot blot analysis of 17 IDDM patients (DM1–19) and 1 control, BML. DNA containing Class II DQ β-sequences from the patients was attached to a filter and hybridized to the radiolabeled oligonucleotide specific for the DQw8 sequence (described above). Positive hybridization signals indicate the patients who carry this DQ β-gene sequence (patients DM1, 2, 4, 7, 10–16, 19). (From Todd, J.A., Bell, J., and McDevitt, H.O.: HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature, 329:599, 1987.)

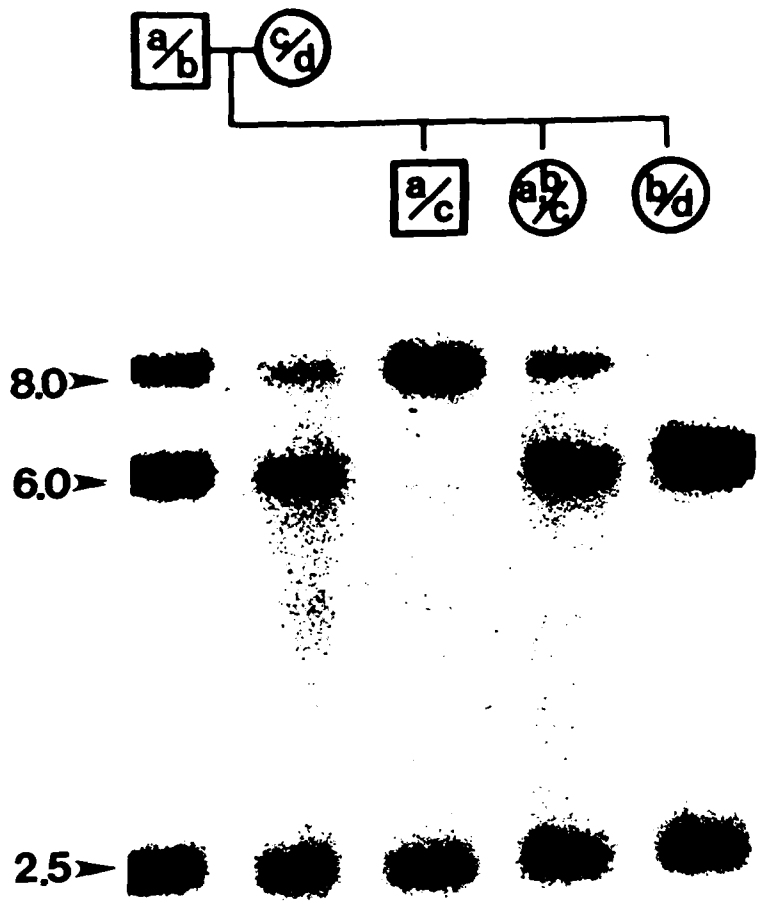

Figure 30–15. The DQw5 allele encoded by the paternal haplotype is present in the HLA recombinant sibling (a, b/c) and sibling a/c. This allele is associated with an 8 kb DNA fragment. The other paternal allele, DQw7, is identified by a 6 kb fragment and is also present in sibling b/d. The maternal allele, DQw5, is present in siblings a/c and a, b/c as an 8 kb fragment. The second maternal allele, DQw7, is present in sibling b/d. This band is 6 kb.

A second method of analysis of Class II genes uses restriction endonucleases to cut DNA into defined fragments. These fragments are separated by size on an agarose gel and are transferred to a membrane. The DNA fragments encoding the Class II genes are detected by hybridization with cloned Class II genes that have been radiolabeled. The genetic region surrounding and encoding the Class II genes is very polymorphic and, when cut with restriction enzymes, gives rise to a polymorphic pattern of restriction fragments that differs among individuals expressing different Class II alleles (Fig. 30–15). These patterns are often complex and difficult to use for identification of Class II alleles. In addition, the majority of restriction enzyme sites are outside of coding regions; thus, differences (or lack of differences) in restriction fragment patterns may not reflect differences (or identity) in the actual coding region sequences. The technique is probably most useful in the detection of DP recombinants when matching siblings for bone marrow transplantation. Because it is difficult to type for DP alleles and recombination between the DP loci and the DR/DQ loci is relatively common, analysis of restriction fragment length polymorphism (RFLP) can be used to detect differences in DP alleles within a family and thus aid in HLA matching for transplantation.

COMPLEMENT TYPING

The complement system is divided into two pathways: the classic pathway contains nine different components totaling at least 12 proteins, and the alternative pathway contains four (see Chap. 33). Three of the 16 proteins are encoded within the MHC and demonstrate inherited structural variants that can be studied by techniques that detect differences in net surface charge due to amino acid differences. Two methods are used to separate proteins: (1) high voltage agarose gel electrophoresis to detect variations of mobility due to charge differences between proteins and (2) isoelectric focusing in thin layer polyacrylamide gels, which demonstrates differences in isoelectric points. Proteins can be visualized by either immunofixation electrophoresis using insolubility of antigen-antibody complexes or by functional overlay gels in which antibody-sensitized sheep

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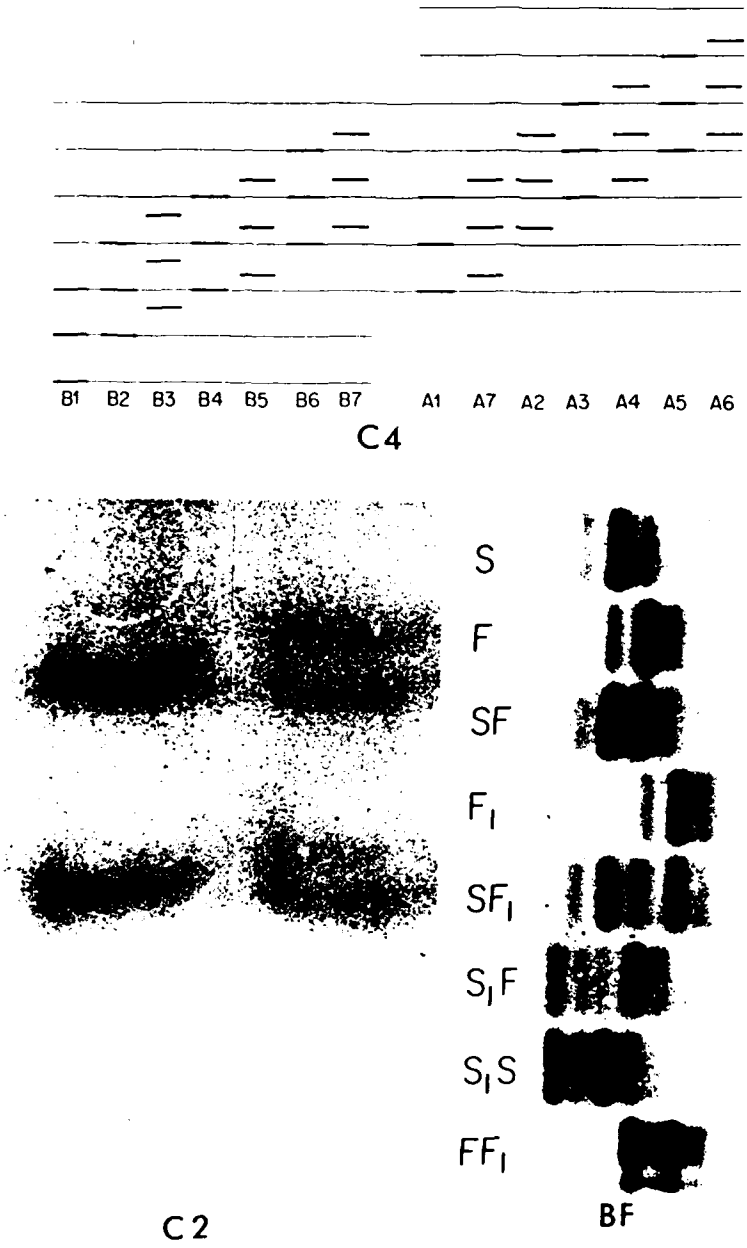

Figure 30–16. Electrophoresis positions of C4 variants relative to one another (diagram). The variants at the C4B (Chido) locus are shown at the left, and those at the C4A (Rodgers) locus are shown at the right. Each gene product consists of three bands. It will be noted that some of the C4B variants correspond closely in position to C4A variants (B7 and A2, for example). The distinction between them is made by use of a C4 sensitive overlay agarose gel in which only C4B variants have appreciable C4 hemolytic activity (Awdeh, 1980). The BQO and AQO (null genes) show no bands.

In the lower portion *(left side)* of the figure, examples of the common C2 type (C) and a heterozygote BC are shown using isoelectric focusing in polyacrylamide gel with agarose gel overlay, containing antibody-sensitized sheep erythrocytes and a *1/90* dilution of normal human serum.

In the lower portion *(right side)* of the figure, examples are shown of electrophoretic patterns of BF variants after agarose gel electrophoresis and immunofixation with anti-factor B antisera. Each gene product consists of one main band and two minor ones. The anode was at the right.

erythrocytes are combined with complement deficient serum (Marcus, 1986).

Proteins from the classic pathway (C2, C4A, and C4B) and one from the alternative pathway (BF) are polymorphic and therefore useful for assignments of MHC haplotypes. In C2 typing, dilute normal human serum can replace C2 deficient serum as a reagent because C2 is the limiting factor in the classic pathway. Patterns are observed after C2-induced hemolysis in overlay gels after isoelectric focusing. The most frequent patterns consist of two major and several minor bands (common pattern). Several rare variants are seen as displacements from the common or C2C variant and one called B (for basic) or A (for acidic).

In C4 typing, the specific proteins are studied by immunofixation electrophoresis, which uses the insolubility of antigen-antibody complexes to visualize the proteins (Fig. 30–16). C4 is the most polymorphic of all complement components with many common alleles at each of its two closely linked loci: *C4A* (acidic) and *C4B* (basic). C4 typing requires three different techniques: (1) Immunofixation electrophoresis after treatment with neuraminidase. The patterns produced show three bands for each variant with some overlap occurring between variants. Additional treatment of the sample with carboxypeptidase reduces each variant to a single band. (2) Detection of C4A versus C4B by functional hemolytic assay. This is to distinguish C4A or C4B overlapping patterns because C4B variants have 5 to 10 times the hemolytic activity of C4A variants. (3) Rodgers (C4A) or Chido (C4B) serologic reactivity. The serum being typed is incubated with either human anti-Rodgers or human anti-Chido to test for inhibition of agglutination with appropriate positive erythrocytes.

Alternatively, C4 variants can be typed for Ch or Rg by immunoblotting. There are two ways to detect null alleles of C4 heterozygotes. Electrophoresis of C4 null (C4A Q0 and C4B Q0) samples demonstrates absence of bands in homozygotes but in heterozygotes requires quantitation by either visual inspection, by crossed immunoelectrophoresis, or by densitometric scanning of the immunofixation patterns. An alternative method is to determine the presence and ratios of the C4A and C4B α-chains after sodium dodecylsulfate polyacrylamide gel electrophoresis of immunoprecipitates.

Properdin factor B (BF) typing is performed after prolonged agarose gel electrophoresis and immunofixation. The homozygous patterns consist of a major band and flanking minor bands and heterozygous patterns are the sum of two homozygous patterns. The major bands are labeled based on the their mobility in relation to the anode: F (fast), S (slow), F1 (faster), and S1 (slower).

TISSUE/ORGAN TRANSPLANTATION

Long-term survival of solid organ and bone marrow transplants represents one of the most challenging goals in medical science. Renal transplantation is a routine procedure in more than 150 major North American medical centers as the therapy of choice for most patients with end-stage renal disease. More recently, bone marrow, heart, liver, and pancreas transplantation is gaining wide acceptance as a therapeutic procedure. The primary obstacle to transplantation is the immunologically mediated rejection of the graft. Allograft rejection is a manifestation of the uniqueness of the individual and is an innate property of the immune system, because the survival of an individual is dependent upon the ability to recognize foreign antigens (be they viral or bacterial pathogens, tumor cells, or, unfortunately, allogeneic tissue) and to respond to them. Therefore, the success of allografts relies on the ability to circumvent the immune reaction, which is accomplished largely by two means: (1) immunosuppressive therapy. Cytotoxic and immunosuppressive agents such as azathioprine and prednisone have been used with moderate success for more than 30 years. Cyclosporin A has become available more recently and is a powerful immunosuppressive agent that has improved the success of all transplantation, but most strikingly non-renal transplants; (2) histocompatibility matching between the donor and the recipient. Optimal histocompatibility matching for HLA increases cadaveric renal allograft survival between 10 to 20 per cent at one year after engraftment and the differences in graft survival between well-matched and poorly matched patients increases incrementally at further time points. In addition, better matching in first transplantations decreases sensitization and thus improves the probability for successful retransplantation following first graft failure. There is also some retrospective evidence that HLA matching for other organs (heart and liver) may be beneficial. For allogeneic bone marrow transplantation, HLA matching of the donor and recipient is absolutely required to achieve any degree of success.

Genetic Basis of Transplantation

The genetic basis of transplantation was first determined in 1916 in mice as a result of tumor transplantation experiments and was subsequently extended to transplants of normal tissue. It was demonstrated that skin grafts within inbred strains that were homozygous for histocompatibility antigens were successful. However, grafts between inbred strains (allografts) were rejected. Grafts from either parent inbred strain to first generation (F1) hybrids survived in all animals, whereas grafts from F1 hybrid offspring to either parent did not survive. The foregoing observations established the laws of transplantation. In 1948, the factors or genes determining the fate of allografts were named "histocompatibility" or "H" genes. Also in 1948, the major histocompatibility locus in the mouse, H-2, was defined by Gorer, who had first described the alloantigens in 1937. There are other histocompatibility or H systems in the mouse, but these have a relatively minor effect on the outcome of grafting and can be overcome by immunosuppression much more easily.

Because convincing evidence already existed in experimental animals that molecules encoded by the MHC represent the major barriers to successful allografting, HLA typing was used to determine compatibility between donor and recipient. Initially, the influence of HLA antigens on graft survival was investigated by Amos and colleagues through skin grafting between family members. As in studies with inbred mice, skin grafts from HLA-identical siblings survived significantly longer than grafts between one-haplotype–matched siblings, parents, or unrelated donors. These observations were extended to and confirmed in renal transplantation during the late 1960s and 1970s.

Within a family, each child inherits one HLA haplotype from each parent in a random fashion; thus, there are only four HLA genotypes possible in a sibship (Fig. 30–3). The probability of occurrence of each genotype is thus one in four. Therefore, approximately 25 per cent of all siblings will inherit the same parental HLA haplotypes and are termed "HLA-identical." Fifty per cent of the siblings will share one HLA haplotype and are termed "haploidentical," and 25 per cent will share no HLA haplotype and are called "haplodistinct." Since offspring inherit one HLA haplotype from each parent, it follows that the parent-child combination will always be haploidentical.

Renal Transplantation

Figure 30–17 shows the 10-year survival of renal allografts from the UCLA registry among the three main categories of donors: (1) HLA-identical sibling; (2) haploidentical parent; and (3) two-haplotype–different cadaver donor. The long-term risk period (greater than

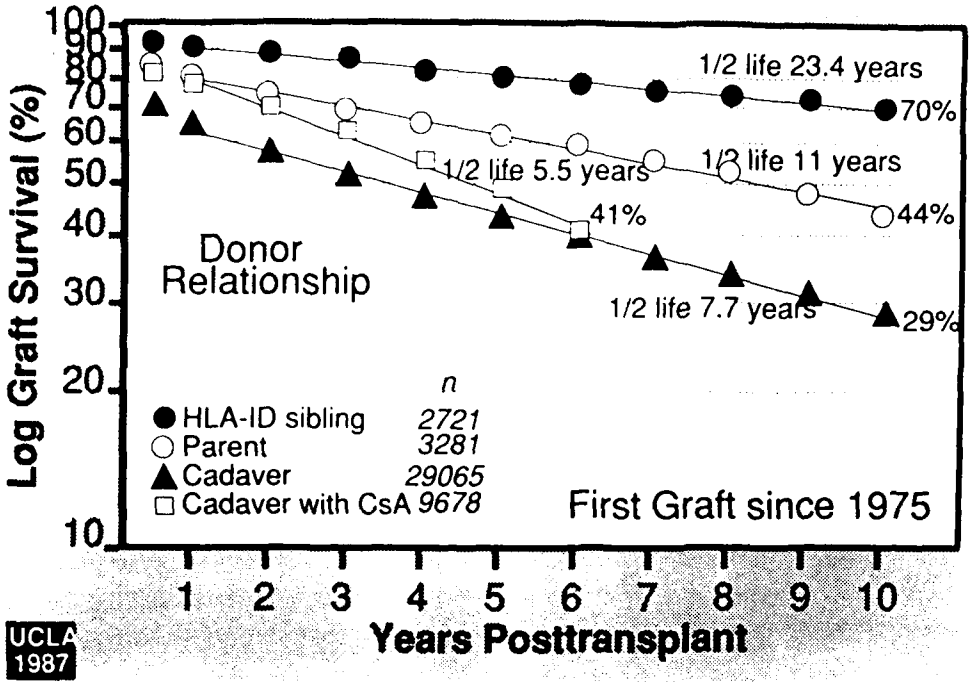

Figure 30–17. Ten-year renal allograft survival of three main categories of histocompatibility. (From Terasaki, P.I., Mickey, M.R., Cecka, M., et al.: Overview. *In* Terasaki, P.I. (ed.): Clinical Transplants 1987. Los Angeles, UCLA Tissue Typing Laboratory, 1987.)

one year post-transplantation) is characterized by a constant failure rate that results in a straight line when plotted on a log scale. This failure rate is characteristic of each category of histocompatibility and the slope of the line has not changed in the past 10 years. Overall grafts between HLA-identical siblings do best (70 per cent at 10 years). Haploidentical grafts survive less well (44 per cent at 10 years). Haplodistinct or two-haplotype–mismatched cadaveric donor grafts survive least well (29 per cent at 10 years) (Terasaki, 1987). Thus, provided medical reasons permit, the order of priorities in living-related allograft donor selection is based on decreasing order of histocompatibility: (1) monozygotic twin; (2) HLA-identical siblings; (3) HLA-haploidentical sibling, child, or parent; (4) first order relatives: grandparents, aunts, uncles, or cousins, preferably haploidentical. Note that a monozygotic twin would also share minor histocompatibility antigens and be the ultimate match, equal to an autograft. Of interest are long-term results with cyclosporin A (CsA) treatment. Although CsA diminishes acute rejection, CsA has no beneficial effect on the long-term survival rate and may prove to be detrimental (Fig. 30–17) because of nephrotoxicity.

The original finding that organ transplants between HLA-identical (and ABO-compatible) siblings survive very well and significantly better than organs transplanted between HLA-mismatched siblings or parents has been consistently confirmed. These data comparing HLA matched and mismatched sibling donors leave no room for doubt that the HLA complex represents a major barrier to successful renal transplantation and is indeed the major histocompatibility complex in man. Compared with results obtained with living-related transplants, the results of matching for HLA in cadaver (unrelated) transplants has not been clear until recently. As definition of HLA antigens has become better refined and as larger data bases have been accumulated, it appears that cadaveric renal allografts that are phenotypically identical for HLA-A, B, and DR (i.e., 0 antigen mismatch) survive as well at three years as grafts from genotypically HLA-identical siblings (Fig. 30–18). In addition, projected 10-year survival of zero A, B, DR mismatched cadaveric donor grafts is only slightly less than for the zero mismatched HLA-identical sibling rate (65 per cent versus 70 per cent). Thus, the United Network for Organ Sharing (UNOS) requires mandatory sharing of zero HLA-A, B, DR mismatched cadaveric kidneys. Transplants with one HLA-A, B, DR antigen mismatch also survive well (predicted 52 per cent at 10 years); however, mismatching for more than one HLA-A, B, DR antigen does not appear to be useful. Since rejection of poorly matched allografts results in highly sensitized patients in whom transplantation is difficult and since second and subsequent transplants overall do less well, the goal should be to provide as many patients as possible with kidneys from zero or one antigen mismatched donors.

The Lymphocyte Cross-match. A positive cross-match between donor lymphocytes and recipient serum is contraindicative to performing that specific transplantation. The cross-match test measures the state of presensitization, if any, in the potential recipient against a specific donor. There is a high correlation of a positive cross-match with hyperacute rejection. This is not surprising, because HLA antigens are found in large amounts in the kidney. There are numerous serologic and cellular methods for analyzing the donor-specific pre-sensitization state; however, complement-mediated lymphocytotoxicity is used conventionally in all HLA

laboratories. This assay, which tests for the presence of cytotoxic antibodies in the serum of the potential recipient that react with lymphocytes of the specific donor, is probably the most important contribution of the HLA tissue typing laboratory to clinical renal transplantation. Final cross-matches should be performed prior to transplantation using a recipient serum sample collected within 48 hours before transplantation if the recipient has pre-existing Class I lymphocytotoxic antibodies or has had a recent sensitizing event such as blood transfusion or transplantation. In the latter case, a serum sample obtained at least 14 days post-alloimmunization should also be used for analysis. Each serum sample should be tested undiluted and at one or more dilutions in duplicate or by more than one technique. Serum samples from potential recipients should be stored at −70°C. and be protected from carbon dioxide and from evaporation. Serum samples used for cross-matching should be retained in the frozen state for at least 12 months following transplantation.

In recent years, the theory and practice of cross-match techniques and interpretation have changed. Currently, the only unequivocal statement that can be made is that a positive T cell cross-match detected by a conventional lymphocytotoxicity technique with a current serum sample that is negative with autologous lymphocytes is contraindicative to transplantation. The majority of antibodies detected by such methods are against HLA-A, B, C antigens. The consequences of a positive cross-match with all other techniques using other cell types are unclear and are currently under investigation. These techniques and the controversies of cross-matching are summarized in the following paragraphs (Ting, 1989).

Historic Positive/Current Negative Cross-match. Previously it was thought that, since memory T cells are long-lived, a positive cross-match with any serum sample (historic or current) was contraindicative of transplantation of an organ from that donor into the recipient. Following a negative cross-match with a current serum sample, many transplant centers now carry out successful transplantation even though there may be a positive cross-match with a historic serum sample (a sample taken more than three to six months pre-transplant) (Cardella, 1982). One of the benefits of this approach is that transplantation candidates with a high level of sensitization that has decreased over time have a higher probability of accepting a transplant. While overall allograft survival has been good following this protocol, there are a few patients who reject their graft in an accelerated fashion. Some centers require that the transplant candidate receive a blood transfusion during those three to six months to identify patients with active suppression of a specific immune response that is likely to be beneficial. One group has shown that transplant recipients who have an uneventful clinical course following grafting exhibited antibodies in the current serum sample that neutralized the donor-specific anti-HLA antibodies found in the historic serum sample (Reed, 1987). In contrast, transplant recipients with a current negative/historic positive cross-match who did not have successful transplantation failed to express neutralizing (anti-idiotypic) antibodies in the current serum sample. Thus, as an adjunct cross-match procedure, the identification of neutralizing antibodies (e.g., anti-idiotypic and/or non-complement fixing) in the current serum sample could potentially identify those donor/recipient pairs that may not reject their transplanted kidney.

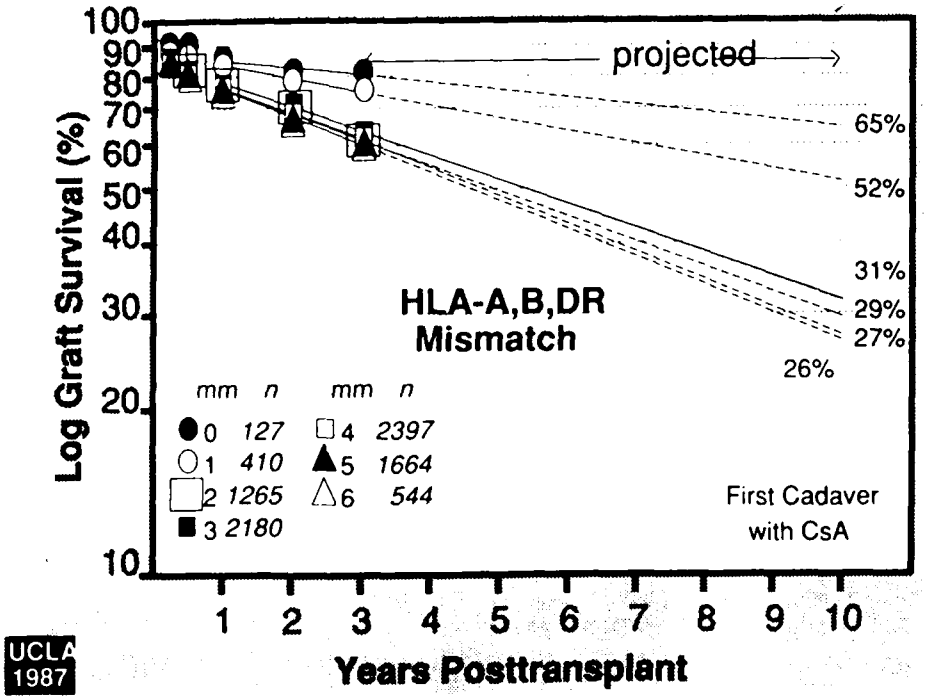

Figure 30–18. Effect of HLA-A, B, or DR mismatching in cadaver donor recipients treated with CsA, projected for 10 years. (From Terasaki, P.I., Mickey, M.R., Cecka, M., et al.: Overview. *In* Terasaki, P.I. (ed.): Clinical Transplants 1987. Los Angeles, UCLA Tissue Typing Laboratory, 1987.)

Autoantibodies. The presence of circulating autoantibodies in the recipient is not harmful to the allograft. Thus an autocross-match should be performed and, if autoantibodies are present, the sera used for cross-matching should be adsorbed to remove the autoreactivity. Some HLA laboratories are using the reducing agent DTE (dithioerythritol) or DTT (dithiothreitol) to differentiate between IgM and IgG antibodies in serum samples because autoantibodies are primarily IgM and HLA antibodies are primarily IgG (Barger, 1989). An aliquot of DTT is added to an aliquot of serum used for cross-matching to inactivate any IgM antibody present. If the DTT-treated serum sample is negative and the untreated sample is positive, some transplantation centers proceed with transplantation. Although the preliminary data are encouraging, DTT is an unsatisfactory alternative to an autocross-match (since not all anti-HLA antibodies are IgG) and, thus, should be used in antibody screening (see later) and as an auxiliary cross-match procedure.

B-Cell Antibodies. Antibodies detected in a B-cell cross-match can be (1) anti-DR/DQ, (2) weak anti-HLA-A,B,C antibodies (B cells have a higher density of Class I molecules and are more sensitive to complement-dependent assays than are T cells), and/or (3) non-HLA antibodies (e.g., autoantibodies). The significance of a positive B-cell cross-match is unclear because many B-cell cross-matches are performed with sera that have not been adsorbed with platelets to remove anti-HLA-A,B,C antibodies (platelets do not express DR/DQ molecules). Although in a number of transplant centers a positive B-cell cross-match is not contraindicative of transplantation, a large study in Minneapolis indicates that a positive cross-match with platelet-adsorbed serum correlates with poor graft survival at two years (Noreen, 1989). In addition, there are eight cases in the literature in which there are early adverse effects of DR-specific, high titered antibodies. B-cell cross-matches should be performed with platelet-adsorbed sera and the data collected for retrospective analysis. Care should be taken to eliminate autoantibodies.

Supplemental Sensitive Cross-match Techniques. The significance of results using highly sensitive techniques to detect low levels of preformed antibodies is unclear, at least for first transplantations. Examples of such sensitive techniques are antiglobulin-augmented cytotoxicity and flow cytometry. Now, possibly because of better immunosuppressive regimens, it appears that results obtained with supplemental sensitive cross-match techniques may not correlate with clinical outcome (incidence of hyperacute rejection, accelerated rejection, and number of rejection episodes) in first transplantations. However, in subsequent transplantations, these sensitive techniques may be clinically relevant. One retrospective study showed that results obtained with the antiglobulin technique may predict those patients who will have more rejection episodes and thus may aid in post-transplantation management. In another retrospective study using flow cytometry, primary non-function was thought to be due to "hidden hyperacute" rejection, which would have been detected by flow cytometric cross-match techniques.

Antibody Screening (Per Cent Reactive Antibody). For renal transplantation candidates, it is mandatory that monthly serum samples be screened for the presence of lymphocytotoxic antibody to determine the state of pre-sensitization of the transplantation candidate. These serum samples also will provide historic samples for use in cross-matching. In addition, it is advisable to identify the most reactive serum sample following rejection and/or nephrectomy of a rejected graft and after blood transfusions. This is determined by testing serum samples obtained at frequent time intervals in the four weeks following the sensitizing event. The presence of lymphocytotoxic antibodies is determined by testing with a well-characterized panel of T cells. A sufficient number of individuals must be included in the cell panel that is used for screening to ensure that any HLA-directed antibody will be detected. As far as possible, the individuals used in this panel should remain the same so that results can be compared from month to month. Results are reported as per cent reactive antibody (PRA). The HLA specificity of the antibody is reported if it can be determined.

Patients with PRAs in excess of 60 per cent have a greatly decreased likelihood of a negative cross-match and, thus, of successfully receiving a transplant. Currently, approximately one third of the transplant candidate pool has a PRA of >60 per cent. The accumulation of large numbers of highly sensitized patients, resulting primarily from rejection of poorly matched allografts, is a major problem because these patients remain on the potential recipient list for long periods of time and are difficult to manage clinically. UNOS distributes to collaborating laboratories trays that contain serum samples from registered transplantation candidates who have high PRAs (>80 per cent). Since sharing of organs from a large pool of donors is required to identify cross-match–compatible kidneys, this permits identification of more favorable recipient candidates among these most difficult-to-transplant patients and decreases the discard rate of shared kidneys because of prolonged ischemia.

Non-renal Organ Transplantation

This includes heart, liver, and pancreas transplantation. Patients and donors should be typed for HLA whenever possible for retrospective analysis. Likewise, monthly serum antibody screenings are recommended to identify the state of sensitization. In most cases, a pre-transplantation cross-match is not practical because of the lengthy testing required. However, whenever possible, it is recommended to prospectively cross-match patients at risk for allograft rejection specifically including those with a PRA of greater than 15 per cent. Retrospective analysis in recipients of both heart and liver transplants suggests that matching for HLA does have a beneficial effect.

Allogeneic Bone Marrow Transplantation

Most bone marrow transplantation is performed for (1) aplastic anemia, (2) leukemia, or (3) genetic disorders such as severe combined immunodeficiency (SCID). These transplants are among the most difficult of all clinical procedures for several reasons. First, at the time of transplantation, the recipients are nearly totally immunodeficient, either because of inherited deficiency (SCID) or because of the pre-transplantation therapy (cytotoxic chemotherapy and irradiation), which prevents the immune system of the recipient from rejecting the donor marrow that is infused several days after pre-treatment. The amount of cytotoxic pretreatment is high enough to eliminate circulating leukocytes, nearly eliminate platelets, and abrogate production of new erythrocytes. Thus, the recipient is profoundly susceptible to all types of infection and would certainly die if not rescued by extraordinary medical care and the allogeneic bone marrow. The second profound risk is the potential of immunologic attack of the recipient by the transplanted marrow resulting in graft versus host disease (GVH). GVH has several forms and is frequently fatal. HLA molecules can act as initiators as well as attack sites for GVH. In spite of these difficulties, a number of transplant centers have achieved exceptional success. In 1988, there were approximately 1600 allogeneic bone marrow transplantations performed in the United States. Recent reports demonstrate extraordinary success with allogeneic marrow transplantion for aplastic anemia (75 per cent survival) and good success for leukemic patients transplanted during their first remission of the disease.

Until recently, it has been generally accepted that only HLA identical sibling donors were acceptable bone marrow donors. However, the success with transplants of slightly mismatched family member donors and HLA matched unrelated bone marrow donors prompted the development of national registries of unrelated bone marrow donors in the United States, England, Canada, and France. In the U.S., there were over 75 unrelated donor transplantations performed during 1988, the first year of national registry operation. Success with these slightly mismatched donors has been similar to success with HLA-identical sibling donors for transplantation of leukemic patients.

Allogeneic bone marrow transplantation may be used, in time, to treat a wide variety of diseases, including genetic and metabolic disorders and immune failure. In addition, it may be used in conjunction with solid organ transplantations. Numerous new approaches are possible, such as the use of pure marrow "stem cells" for transplantation and the ability to place corrective genes into autologous cells to correct disorders such as thalassemia and sickle cell disease. The availability of growth factors that influence the growth and regulation of hematopoietic cells provides new tools to improve bone marrow transplantation. If the problems surrounding this most difficult procedure are overcome, bone marrow transplantation may become one of the most widely used methods for the treatment of a variety of diseases.

The bone marrow pre-transplantation workup includes HLA-A, B, and DR typing of all available members of the immediate family to establish inheritance of haplotypes. HLA-D, HLA-DP, HLA-DQ, complement, and GLO typing may be appropriate. MLC testing is mandatory to confirm identity. The MLC must be performed with lymphocytes from the donor and the patient used as both responder and stimulator (e.g., in both directions). Cross-match tests (patient serum + donor cells and donor serum + patient cells) are advisable.

ROLE OF MHC MOLECULES AS IMMUNE RESPONSE GENES

Much of what is known about the genetic control of immune responses comes from the study in laboratory animals, particularly the mouse and guinea pig, of the antibody response to simple antigens, such as synthetic polypeptides. Attempts to study the human antibody response to very similar antigens have been difficult for several reasons. Many individuals have a delayed skin response to these polymers, even when there has been no known prior exposure. Furthermore, there is no correlation between the skin reaction and the proliferative response of lymphocytes from the same individual on exposure *in vitro* to the polypeptides. Nevertheless, in several studies of immune responses *in vitro* to synthetic polypeptides, subjects could be classified into high, intermediate, and non-responder phenotypes. Family studies indicated that high responses were inherited as dominant HLA-linked traits, but it was not possible to show linkage disequilibrium with specific HLA types of haplotypes.

Studies of total IgE level and antibody titers to the minor ragweed allergen Ra3, in both allergic and nonallergic populations, at first suggested polygenic control. Some investigators proposed that high levels of IgE are determined by two recessive alleles. Others found polygenic heritability, with evidence for a major regulatory locus. Reanalysis of the data by another method did not confirm this finding, however. Analysis of IgE levels in families suggested heterogeneity among the families. Hence, it appears that inheritance of the regulation of IgE basal level is not HLA-linked and is unclear. HLA associations with such allergic diseases as asthma, hay fever, urticaria, and eczema have been sought but have not been found. Studies with purified aeroantigens, such as ragweed antigen E, Ra3, and Ra5, have included a population study of IgE-mediated skin responses to the low molecular weight allergen Ra5, in which an association with the HLA-B7 cross-reacting group was shown. There appeared to be an association of ragweed hay fever, intense skin reactivity to antigen E, and HLA haplotypes in some families with ragweed hay fever. It appears that there may be a gene for ragweed sensitivity linked to HLA in some families, while

IgE levels are controlled by a second, non-HLA-linked locus (or loci). Atopic patients showed an association with HLA-DR2. It appears that this association prognosticates response to allergen immunotherapy. In a study of MHC haplotypes in a series of patients and their families with ragweed pollen allergy, it was found that the extended haplotype [HLA-B7, SC31, DR2] or its fragment, SC31, DR2 marked high IgE anti-Ra5 levels. The latter, in turn, predisposed to asthma rather than rhinitis alone.

It has been generally accepted that MHC Class II gene products are involved in helper T-cell function. However, certain antigens have been implicated in the generation of suppression of immune responses by Class II gene products. For example, the *in vitro* IgE response to cedar pollen antigen is suppressed in individuals bearing a single dose of HLA-DQw3 and low responsiveness to streptococcal wall antigen is associated with HLA-DQw1. Also, published experiments suggest that suppression is blocked by DQ-specific monoclonal antibodies, whereas DR-specific monoclonal antibodies prevented the *in vitro* response.

The response *in vitro* of human lymphocytes to streptokinase/streptodornase revealed a significant association with HLA-B5, and there was also an association between HLA-Cw3 and a low response *in vitro* to vaccinia virus. Reports of the antigen-specific T-cell proliferative response *in vitro* to streptococcal cell wall antigen and to Schistosomal antigen demonstrated that low response was dominant, and that low response to Schistosomal antigen is associated with the HLA haplotype HLA-Bw52, Dw12, DR2 in Japanese subjects. Also, the expression of low responsiveness to streptococcal wall antigen was controlled by an immune suppressor gene that is HLA-linked and controls the generation of antigen-specific suppressor T cells.

A highly significant ($p < 0.0001$) association was noted between HLA-DR3 and the presence of antibodies to native DNA, not only in patients with systemic lupus erythematosus but also in non-lupus individuals. HLA-DR3 and anti-DNA antibody may distinguish a clinical subgroup of systemic lupus erythematosus from another subgroup with HLA-DR2 and anti-SM antibody. Japanese patients with high titers of anti-DNA antibody showed a strong association with HLA-Bw35, although SLE patients as a whole do not.

The HLA markers have been useful in the investigation of virus-host interaction in two general ways: (1) analysis of the restriction phenomenon by allele modification and (2) the study of the genetics of the immune response. For example, HLA restriction was found in the target-effector cell lysis of influenza virus–infected cells. On the other hand, in studies of both monozygotic and dizygotic twins, the antibody response to measles vaccine and diphtheria toxoid correlated directly with the number of shared HLA haplotypes.

More important for this discussion is the fact that IgA deficiency (it occurs in about one in 700 apparently healthy blood donors) is associated with B8 and DR3. Also, an association between HLA-B8 and immunization against the platelet-specific antigen Zw was found. Raum (1981) and Svejgaard (1983) have reported extensively on the role of MHC molecules as immune response genes.

Possible associations of the HLA system with adverse reactions to drugs have seldom been explored. However, gold and D-penicillamine nephropathy have been associated with HLA-B8 and HLA-DR3 and hydralazine SLE with HLA-DR4, thus suggesting that HLA plays an important role in drug-induced autoimmune disease. Also, the frequency of HLA-B44 was significantly increased among psychiatric patients who developed autoantibodies after chronic treatment with chlorpromazine (Canoso, 1986). More recently, we have observed a bimodal response to the hepatitis B vaccine. Among hypo- and non-responders there was an increase of homozygotes for the extended haplotype [HLA-B8, SC01, DR3]. We prospectively minimized homozygotes and heterozygotes and showed a markedly reduced antibody response in the homozygotes but not the heterozygotes. This is clear-cut evidence for the absence of a "normal" dominant immune response gene on this haplotype (Alper, 1989).

MHC-DISEASE ASSOCIATIONS

Diseases Involving Mendelian Inheritance of Defects in MHC Genes

Before turning to the bulk of MHC-associated diseases with their uncertain modes of inheritance and probable polygenic contribution, it is instructive to consider three disorders involving Class III genes. The disorders are C2 deficiency, C4 deficiency, and congenital adrenal hyperplasia due to 21OH deficiency. In addition to these diseases being of intrinsic interest, it is highly instructive to analyze other MHC markers in these relatively straightforward diseases. We need all the clues we can get before attempting to deal with the likes of rheumatoid arthritis and Type I diabetes mellitus.

C2 Deficiency. Deficiency of the second component of complement has been reported only in Caucasians and it is the most common complement protein deficiency state in that ethnic group. About 40 per cent of reported cases have a systemic lupus-like disease; however, only 16 per cent of the homozygous C2 deficient siblings of these probands have had the lupus-like disease. This strongly suggests ascertainment bias. Nevertheless, there clearly is an increased tendency of homozygous C2 deficient subjects to have lupus-like disorders, perhaps related to defective clearance or disaggregation of immune complexes. Complete C2 deficiency results from homozygosity for a null allele, *C2*Q0*, which has a frequency of slightly less than .01. There is approximately one homozygote per 10,000 individuals. Almost all patients have elements of an extended haplotype [HLA-(A25), B18, SO42, DR2] containing the C2 deficiency gene. The complotype associations are most frequent, followed by the DR, HLA-B, and HLA-A associations, in keeping with the known gene order. The

exact distributions suggest that this form of C2 deficiency arose as a mutation in a C2 gene in a Caucasian with the aforementioned haplotype between 650 and 1325 years ago. This is supported by the observation that no homozygous C2 deficient individual has had any other extended haplotype.

C4 Deficiency. Most (11/17) patients with complete deficiency of C4 have systemic lupus erythematosus and an additional two patients have either discoid lupus or anaphylactoid purpura. This susceptibility probably also relates to defective handling of immune complexes, as in C2 deficiency and other early acting complement component deficiencies. Because there are two loci encoding C4, complete deficiency results from the inheritance of two double null C4 haplotypes *(C4A*Q0, C4B*Q0)*. In contrast to C2 deficiency, C4 deficiency has been reported on at least nine different MHC haplotypes, including those with different BF types, suggesting that the deficiency arose from a number of different mutations. Double null C4 haplotypes appear to be quite rare and can be detected only in family studies. The level of C4 in serum is extremely variable and cannot be used reliably to detect heterozygotes for complete deficiency, even though there is a rough correlation between the level of C4 and the number of expressed C4 genes. In addition to the recessive MHC-linked complete form of C4 deficiency, one family has been described in which severe but not complete C4 deficiency is inherited as an autosomal dominant trait unlinked to the MHC. It is possible that the defect in this family lies in a C4 regulatory gene. There is no apparent connection between this form of incomplete C4 deficiency and any disease.

21OH Deficiency, Congenital Adrenal Hyperplasia. This disorder is clinically heterogeneous, with a severe salt-wasting form, a milder late onset form manifested largely by masculinization in girls, and a mild cryptic form. The MHC linkage of this disorder was discovered before any MHC associations were detected and before it was known that the 21OH loci were located in the MHC. Subsequently, it was found that in European Caucasians studied in the northeast U.S. and in England, 20 per cent or more of haplotypes in patients with the salt-wasting form were the rare extended haplotype [HLA-A1, Cw6, Bw47, FC91,0, DR7, GL01]. This haplotype appears to be very common in the Midlands of Britain, and, thus, it is likely that a mutation leading to the disease probably occurred on a closely related haplotype in an individual from this region. It has been shown that this haplotype has a deletion of both C4B and 21OHB, thus explaining the severity of symptoms and complete deficiency of the enzyme in homozygotes for this haplotype. It is of interest that a similar haplotype without the deletion has been found in the Amish population. Among patients with milder and cryptic disease, a different extended haplotype is common: [HLA-B14, SC2(1,2), DR1]. This haplotype is common, particularly in southern Europe, and has a frequency in Caucasians in Boston of over .01. No patient homozygous for 21-hydroxylase deficiency has the most common of Caucasian extended haplotypes [HLA-B8, SC01, DR3]. In all forms of 21OH deficiency, the bulk of MHC haplotypes are not extended and there is a great variety of complotypes suggesting that many independent mutations have led to either deletion or derangement of the 21OH genes. The study of 21OH deficiency in a Venezuelan mestizo population revealed none of the haplotypes found in Boston Caucasian patients. Other marker haplotypes different from ethnically matched controls were present, however. In Australia, the haplotype HLA-Bw22, SB45 was found to be increased among patients with 21OH deficiency. Healthy persons who carried this haplotype but who had no family history of 21OH deficiency could be shown to be carriers of the defect.

From these observations, we can attempt to extract some general principles for possible application to those diseases of unknown inheritance and pathogenesis that show MHC association or linkage. First, if a disease susceptibility gene occurs on an extended haplotype, all the alleles of that haplotype will show positive associations with the disease. Conversely, if it does not occur on an extended haplotype, all of the alleles on that haplotype will show a negative association with the disease. Because extended haplotypes have fixity of DNA and independent examples resemble each other closely, all examples of a disease-associated extended haplotype, even in healthy persons, probably carry a susceptibility gene. All disease markers, and particularly extended haplotypes, show a high degree of ethnic specificity. It is therefore particularly important in studies of MHC markers for disease to compare disease alleles and haplotypes (those in patients) with those in carefully ethnically matched controls. Ideally, ethnic matching should include specific regions of origin or ethnic subgroups. Finally, if markers for a disease are parts of extended haplotypes, the susceptibility gene may be anywhere in the region of fixed DNA.

Diseases of Unknown Etiology and Pathogenesis

A remarkable number of diseases have been reported to show MHC associations. These disorders are very different from one another, and although most have at least some immunologic aspects, a few do not. There are many problems that confound our attempts to understand the mechanisms by which these diseases occur, and analysis of MHC markers in patients and their families has helped only marginally to clarify the picture. The greatest source of confusion is a phenomenon termed "incomplete penetrance." This is seen most clearly in monozygotic twins who presumably have identical genes. If one such twin has one of these diseases (Type I diabetes mellitus, for example), the other twin does not necessarily have the disease. For Type I diabetes, the concordance rate appears to approach no higher than 50 per cent. This suggests that "penetrance" of a disease in a completely susceptible host is incomplete. Although there is excellent reason to consider genes in the MHC as determinants of Type

I diabetes susceptibility, only 15 per cent of families with MHC-identical siblings of patients have insulin-dependent diabetes. This is strong evidence for the influence of genes at a second, non–MHC-linked locus (or loci) in determining susceptibility. These considerations make the likelihood small of finding families with more than one affected member in one generation or in over two or more generations. Yet it is just such patterns that ordinarily allow us to decide on modes of inheritance. Other complicating factors are our inability to determine whether we are studying a group of patients homogeneous in terms of genetic determination. About 5 or 6 per cent of random families with a diabetic proband will have a second affected child. Of these sib pairs, approximately 60 per cent will be MHC identical, 35 per cent will be haploidentical, and a few per cent will share no MHC haplotypes. This pattern suggests recessive inheritance of an MHC-linked susceptibility gene for the disease. This conclusion is also supported by the results of analysis of the distribution of homozygotes and heterozygotes for *BF*F1* among 1100 Type I diabetics. Nevertheless, the departure from 100 per cent MHC identity of affected sibs has to be explained. Among the explanations are crossing over between the susceptibility locus and the MHC, impenetrant susceptible parents, and heterogeneity of mode of inheritance, including variable penetrance of homozygotes and heterozygotes. This has led to a vast plethora of disease models with no way to distinguish among them. At the very least, however, family studies provide highly useful haplotype data and usually allow the assignment of homozygosity for an HLA marker in probands. They also have established that MHC association in most of the diseases of interest is based on linkage between a susceptibility gene and the MHC.

Yet another unknown is the number of different susceptibility alleles for a disease in any specific population. In this regard, extended haplotypes can be helpful because, if they are increased in patients, they probably represent a single susceptibility allele that could be anywhere in the region of fixity. However, some patients have only portions of these haplotypes and this provides clues to the location of specific alleles involved. Another clue may be sharing of specific alleles by two or more different extended haplotypes.

Specific Diseases Without Evidence of Mendelian Inheritance and with MHC Associations. Table 30–8 gives some MHC-associated diseases and their markers. The list is not exhaustive and we shall discuss only a few diseases on it by way of illustration. The application of methods of analysis already cited has aided in understanding of only a few of these disorders. For example, the distribution of HLA-B27 in patients with ankylosing spondylitis strongly suggests dominant inheritance, even though most individuals who carry HLA-B27 do not have the disease. Further, it has been shown that there are a number of molecularly distinguishable forms of HLA-B27 and all of these are increased among patients, suggesting that a common B27-associated determinant is involved. This is also supported by the observation that there is no specific extended haplotype with HLA-B27 increased among patients.

In the non-immunologic disease idiopathic hemochromatosis, there is good evidence for recessive inheritance with some expression of iron transport anomalies in heterozygotes. There is also excellent evidence that the disease is closely linked to the telomeric side of the HLA complex and that there are associations with HLA-A3 and, to a lesser extent, B14 and B7.

The most studied of all MHC-linked diseases is Type I diabetes mellitus. Some studies have already been mentioned. There are striking increases in HLA-DR3 and DR4 in Caucasian patients. Heterozygotes for DR3/DR4 are increased in frequency compared with the expectations of the Hardy-Weinberg equilibrium. The reasons for this are unclear. It has been suggested that the presence of aspartic acid at position 57 in the first variable domain of the DQB chain is protective against the disease (Todd, 1987). In susceptible haplotypes, this position is most frequently valine or serine. In family studies, it was shown that all of the DR3 increase in patients was the result of the increase in the extended haplotypes [HLA-B8, SC01, DR3] and [HLA-B18, FIC30, DR3] and much of the increase in DR4 resulted from an increase in [HLA-B62, SC33, DR4] and [HLA-B38, SC21, DR41, but there was also an increase in non-extended HLA-DR4 haplotypes. By DNA analysis, the increase in DR4 is primarily in that subset of DR4 in linkage disequilibrium with HLA-DQw8 that marks the two extended haplotypes (Ahmed, unpublished report).

There is good evidence for recessive inheritance of an MHC-linked susceptibility gene for gluten-sensitive enteropathy. In Caucasians, two extended haplotypes account for over 60 per cent of haplotypes in patients: [HLA-B8, SC01, DR3] and [HLA-B44, FC31, DR7]. Analogously to diabetes, there is an increase in HLA-DR3/DR7 heterozygotes over either homozygote and not all of the increase in DR7 is explained by the increase in the extended haplotype that bears it. There is also evidence that there is a susceptibility gene linked to the immunoglobulin heavy chain gene complex on chromosome 14.

Pemphigus vulgaris is said to be particularly common in the Jewish population. There is a striking increase in HLA-DR4, which is entirely attributable to two related extended haplotypes in that ethnic group: [HLA-B38, SC21, DR4, DQw8] and [HLA-B35, SC31, DR4, DQw8]. In contrast, non-Jewish patients express the extended haplotype [HLA-Bw55, SB45, DRw6, DQw5] DRw6, DQw5 alone or DR4, DQw8 alone. This suggests that two mutations gave rise to MHC susceptibility genes for pemphigus vulgaris. One, perhaps the more ancient one, arose in an ancestor of the Jewish population on a precursor extended haplotype [HLA-B38/35, SC21,SC31, DR4, DQw8] and diffused through recombinants into non-Jewish populations. The second mutation occurred in an individual expressing the [HLA-Bw55, SB45, DRw6, DQw5] extended haplotype, possibly in Italy (most patients with the haplotype are Italian), and diffused to other populations as recombinants (Ahmed, unpublished report).

Rheumatoid arthritis (RA) with onset in childhood differs clinically from juvenile rheumatoid arthritis

(JRA). In recent years, attempts have been made to subdivide JRA into several distinct syndromes based on the mode of onset. Three main groups are (1) systemic with daily intermittent fever, (2) polyarticular, and (3) pauciarticular arthritis. The pauciarticular group is further subdivided into (1) persistent pauciarticular, (1a) males with onset over the age of eight, (1b) a form with iritis, and (2) conversion to polyarticular. The soundness of this clinical classification has been borne out by studies of HLA association and long-term follow-up. In contrast to the DR4 association with RA discussed earlier, the HLA association with JRA is quite different. Furthermore, other HLA-D and DR associations vary with the JRA subsets. Male patients with onset after age eight have a markedly increased frequency of HLA-B27. Early onset pauciarticular JRA and iritis are associated with HLA-DR5, DRw8, and in some patients DRw6.

A number of causes of renal failure are mediated by immunologic injury. Antibodies directed at glomerular basement membrane mediate Goodpasture syndrome, which is associated with HLA-DR2. HLA-DR2 and HLA-DR3 as well as *C4A*Q0* have been found to be elevated in systemic lupus erythematosus, the latter two markers perhaps as part of the extended haplotype [HLA-B8, SC01, DR3].

Elements of the extended haplotype [HLA-B8, SC01, DR3] have been reported to be increased in a wide variety of diseases in addition to those already mentioned, including dermatitis herpetiformis, chronic active hepatitis without HBsAg, Graves' disease, and myasthenia gravis. It may be that this haplotype functions as a genetic sink, accumulating disease susceptibility alleles, because of some present or previous selective advantage and a difference in structure compared with other haplotypes that restricted crossing over with the general pool of haplotypes.

Methods of Detecting Association or Linkage of Disease with Genetic Markers

The Strength of an Association. Several methods have been devised for detecting the degree of association of genetic markers with hypothesized genes for disease susceptibility. One is to compute the risk of disease among individuals carrying a specific allele of a polymorphic system. In this computation, relative risk (RR) calculates the risk of carrying a marker in a population of diseased individuals compared with a control population. More interesting is an estimate of the risk of having disease in an individual carrying the marker, but this is much more difficult to estimate (Tables 30–8 and 30–9).

This strength of association is the delta of Bergston and Thomson (Svejgaard, 1983), which is the same as the etiologic fraction (EF) of Miettinen (1976). If, in a patient population, "a" individuals carry the specific character but "b" individuals do not and, in the control (normal) population, "c" individuals carry the character, the information can be conveniently written in the 2 × 2 table:

NUMBER OF INDIVIDUALS

	Character Positive	Character Negative
Patient	a	b
Control	c	d

The frequency of the character in this patient population (h_p) is

$$h_p = \frac{a}{a + b}$$

The relative risk (RR) is defined as

$$RR = \frac{a \times d}{b \times c}$$

Etiologic fraction (EF) (Miettinen, 1976) is defined as

$$EF = \left(\frac{RR - 1}{RR}\right)\left(\frac{a}{a + b}\right)$$

$$= \frac{RR - 1}{RR} \times h_p$$

Similarly, in decreased risks, for which the RR is less than 1, the preventive factor (PF) can be used.

$$PF = \frac{(1 - RR)(h_p)}{RR(1 - h_p) + h_p}$$

The EF and PF fractions can vary between 0 (no association) and 1.0 (maximal association).

Sib Pair Analysis. This method was introduced to overcome the problems of incomplete penetrance of diseases and variations in age of onset (Penrose, 1935). The method is based on the assumption that if HLA and/or genes closely linked to HLA have no influence on the development of a disease, then the affected sib pairs will share HLA haplotypes with a normal frequency: 25 per cent will share both haplotypes, 50 per cent will share one, and 25 per cent will share no haplotypes. Thus, observed and expected distributions of haplotype sharing are compared. Once the mode of inheritance has been established, the "disease" gene frequency and penetrance can be established (Thomson, 1977).

Analysis of Mode of Inheritance Based on Population Studies. Thomson and Bodmer (1977) have devised a method for analyzing population data for markers closely linked to susceptibility loci for diseases with incomplete penetrance. In essence, this method predicts the proportion of homozygotes, heterozygotes, and non-carriers for the linked marker expected in the cases of dominant or recessive inheritance. The greatest difference between the two modes of inheritance is obtained in the proportion of individuals who are homozygous for the marker. Application of this method to HLA-B27 and ankylosing spondylitis led, on statistical grounds, to rejection of a recessive mechanism. Thus, it was concluded that susceptibility to ankylosing spondylitis is inherited as a dominant trait.

Similarly, the same method of analysis was applied

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Table 30–8. EXAMPLES OF ASSOCIATION BETWEEN HLA AND DISEASE

Disease	HLA Antigen	Relative Risk (RR)*	Etiologic Fraction (EF)†
Arthropathies			
Ankylosing spondylitis	B27	87.4	0.89
Reiter syndrome	B27	37.0	0.77
Rheumatoid arthritis	DR4	4.2	0.38
Endocrine diseases			
Juvenile insulin-dependent diabetes	D/DR3	3.3	0.39
	D/DR4	6.4	0.63
	D/DR2	0.2	
Graves' disease	D/DR3	3.7	0.42
Idiopathic Addison's disease	D/DR3	6.3	0.58
Eye diseases			
Acute anterior uveitis	B27	10.4	0.47
Optic neuritis	D/DR2	2.4	0.27
Inflammatory disease			
Subacute thyroiditis	B35	13.7	0.65
Intestinal disease			
Celiac disease	D/DR3	10.8	0.72
Liver disease			
Chronic autoimmune hepatitis	B8	9.0	
Neurologic disease			
Multiple sclerosis	D/DR2	4.1	0.45
Skin diseases			
Psoriasis vulgaris	Cw6	13.3	0.81
Pemphigus (Jews)	D/DR4	14.4	0.81
Dermatitis herpetiformis	D/DR3	15.4	0.80
Behçet's disease	B5	6.3	0.34
Systemic diseases			
Myasthenia gravis	D/DR3	2.5	0.30
	B8	2.7	0.30
Sicca syndrome	D/DR3	9.7	0.70
Systemic lupus erythematosus	D/DR3	5.8	0.58
Idiopathic hemochromatosis	A3	8.2	0.67
	B14	4.7	0.13
Goodpasture syndrome	D/DR2	15.9	0.82
Idiopathic membranous nephropathy	D/DR3	12.0	0.69

*RR = relative risk. Calculated by $\frac{a \times d}{b \times c}$, where a and b are the numbers of individuals with character present or absent in the patients and c and d the characters present or absent in the control population.

†EF = etiologic fraction = $\frac{(RR - 1)}{(RR)} \frac{(a)}{(a + b)} = \frac{(RR - 1)}{(RR)} (hp)$

Table 30–9. RELATIVE RISKS (RR)* FOR MHC ALLELES IN SEVERAL DISEASES

	HLA-A		HLA-B		HLA-DR		BF	
Disease	*Allele*	*RR*	*Allele*	*RR*	*Allele*	*RR*	*Allele*	*RR*
Juvenile onset insulin-dependent diabetes mellitus	A1	1.6	B8	2.5	DR3	4.5		
			B15	2.5	DR4	4.5		
			B18	2.5			BFF1	8
			B7	0.1	DR2	0.1		
Multiple sclerosis	A3	1.8	B7	2.5	DR2	4.2		
Gluten enteropathy	A1	1.8	B8	8.0	DR3	17		
Chronic active hepatitis	A1	1.7	B8	3.0	DR3	2.2		
Idiopathic membranous glomerulonephritis			B8	2.3	DR3	4.4		
			B18	2.3			BFF1	16
Idiopathic hemochromatosis	A3	4.8	B7	1.9			BFF	2.0
	A1	2.0	B14	4.9	DRw6	3.1		
			B15	3.5	DR4	2.9		

*RR = $\frac{\text{patients with marker}}{\text{patients without marker}} \times \frac{\text{controls without marker}}{\text{controls with marker}}$

to the distribution of *BF*F1* among 1107 patients with insulin-dependent diabetes mellitus (IDDM). For dominant inheritance, 1.89 homozygotes were predicted, and for recessive inheritance 6.2. Seven *BF*F1* homozygotes were found, a result consistent only with recessive inheritance. Other modes of inheritance that could be rejected by these observations include simple dominant, epistatic (disease resulting from the presence of non-allelic genes), or overdominant (disease with greater penetrance when two specific alleles are present than when other combinations, including homozygosity for each specific allele, occur). Although a mixed model with different penetrance for homozygotes and heterozygotes could not be completely ruled out, other considerations make such a model unsatisfactory.

Lod Score Method. This is a statistical measure of linkage between a marker locus such as MHC and a disease susceptibility gene: (1) the Z value is the ratio of the maximum likelihood of finding linkage ($P(F_1/\text{theta})$) to that of no linkage at a particular recombination value theta; and (2) the theta (θ) value or recombination frequency, which is a measure of distance from a given locus corresponding to maximum Z value. The lod score expresses the probability that alleles at two loci will segregate together, in terms of the ratio between the observed recombination frequency (theta), and 0.50, the recombination frequency for alleles that assort independently. Various values of (θ) from 0 to 0.5 are substituted in the equation:

$$P(F_1/\theta) = \tfrac{1}{2}[\theta^r(1-\theta)^{n-r} + \theta^{n-r}(1-\theta)^r]$$

where n = the number of children in a given family and r = the number of recombinants. The probability of obtaining a pedigree for a given value of theta (recombination frequency from 0 to 0.5) is expressed as the ratio of $P(F_1/\text{theta})$ in a given family at a given recombination fraction theta (from 0 to 0.5) to $P(F_1/0.5)$ in the same family assuming no recombination that can be expressed as the lod score (Z):

$$z = \log_{10}\frac{P(F_1/\theta)}{P(F_1/0.5)}$$

and Z = sum of all z values. Values of Z greater than zero favor linkage and those less than or equal to zero are against linkage. In general, a Z value greater than 3 (for some values of 0 less than 0.5) means that the odds in favor of linkage are 1000 to 1 (probability 1/1000, as opposed to no linkage or independence). It is easier to calculate linkage for co-dominant traits, i.e., HLA and GLO, than for recessive traits. In studies of linkage of HLA and disease, the parents may have recessive susceptibility or a dominantly expressed impenetrant susceptibility gene. In such cases, the presence of the disease in the children may determine the likelihood of transmission by the father or mother (dominant) or both (recessive) (Sutton, 1980). The calculations of lod are informative, but they have inaccuracies such as difficulty of distinguishing recombinants from impenetrant susceptible parent or sibling.

POSSIBLE MECHANISMS FOR MHC AND DISEASE ASSOCIATION

There are a number of diseases in which specific alleles of various MHC loci occur at higher frequencies in patients than in normal control populations. These associations may have arisen in several ways: inbreeding, population stratification, or linkage. The Fifth International Histocompatibility Workshop in 1972 demonstrated that most antigens of the HLA system vary considerably in frequency at different geographic locations. These differences could be in the frequency of individual alleles or in extended haplotypes. For example, in pemphigus vulgaris, [HLA-B38, SC21, DR4] is increased in Jewish patients, whereas [HLA-Bw55, SB45, DRw6] is increased in non-Jewish patients. In both cases, it is necessary to compare the patients with ethnically matched controls. These results emphasize the necessity of selecting control populations carefully for comparison with the gene frequencies observed in the disease populations. It appears, however, that at least some of the observed associations do represent linkage of a disease susceptibility locus to the MHC or else arise from an interaction of MHC antigens with environmental factors, initiating a pathologic process. A number of possible mechanisms for such effects have been suggested, many of which derive from experimental study of the mouse. Because the number of associations already detected is large, it seems probable that more than one of the proposed mechanisms are operative. The traditional mechanisms postulated are as follows:

Altered Self-Antigens

As previously discussed, it appears that T cells demonstrate commitment to the MHC and that H-2 and HLA antigens probably play a role in immune surveillance. Jerne (1971) and Benacerraf (1978) have proposed that functionally mature T cells have low reactivity for autologous MHC antigens and concomitantly high affinity for allogeneic antigens. This model predicts that clones of alloreactive T cells should be highly cross-reactive with modified syngenetic cells. The reactivities were shown to be dependent on the H-2D and H2-K type of the host. Similarly, Zinkernagel and Doherty (1974) have demonstrated that the immune response to virus-infected cells is restricted to cells bearing the same altered H-2D or H2-K molecules. In a family study in man, cytotoxic T-cell responses to influenza virus–infected autologous cells *in vitro* showed T-cell recognition of influenza virus (by cytotoxicity) that was dependent upon HLA type (McMichael, 1977). This restriction can be demonstrated with isolated HLA antigens reconstituted into phospholipid vesicles (Engelhard, 1980). Accordingly, a factor has been identified in *Klebsiella pneumoniae* culture filtrates that modifies either HLA-B27 or a closely associated cell surface component (Geczy, 1981). This modifed HLA-B27 induces effector cells capable of injuring specific target tissues (e.g., synovia and cartilage) bearing "al-

tered self" determinants. The destruction of appropriate target cells may trigger a complex chain of events leading to ankylosing spondylitis or uveitis. Reactive arthritis may also follow *Yersinia* or *Shigella* infection, but these organisms have not been shown to alter antigen specificities. Among patients with ankylosing spondylitis, reactive arthropathies, and acute anterior uveitis, about 90 per cent of Caucasian patients, as compared with 5 to 10 per cent of healthy controls, have B27. The association of B27 with these syndromes in four racial groups (Caucasians, blacks, Japanese, American Indians) suggests that B27 itself or a gene closely linked to B27 is involved in the pathogenesis of these diseases.

Another example of MHC and disease association that may be explained by some interaction between an HLA antigen and an etiologic agent is gluten-sensitive enteropathy. Organ cultures in patients with active gluten-sensitive enteropathy demonstrated that gluten exerts a toxic effect on intestinal mucosa, inhibiting the epithelial cell maturation. This effect was seen more frequently in HLA-B8 positive patients with active gluten-sensitive enteropathy than in HLA-B8 negative patients (Falchuk, 1980).

Molecular Mimicry

It is postulated that in certain cases HLA gene products may resemble or be closely related in structure to an antigen of an infective agent, hence rendering the system unresponsive because of cross-tolerance. This mechanism has been invoked to explain both the HLA-B27–associated diseases and certain other diseases with strong HLA-B associations: Behçet disease (HLA-B5) and de Quervain subacute thyroiditis (HLA-B35). A number of bacterial antigens have been examined for cross-reactivity with human HLA antigens. Epidemiologic studies have demonstrated a temporal relationship between bowel infections by *Klebsiella pneumoniae* and other enteric bacteria *(Shigella, Yersinia)* preceding the onset of ankylosing spondylitis (AS). Also, serologic cross-reactivity between certain strains of *Klebsiella* and cells expressing HLA-B27 has been found. A sharing of an epitope by HLA-B27 and a *Klebsiella* surface antigen may elicit an autoimmune response involved in the pathogenesis of AS (reviewed by Keat, 1986). A sequence of six consecutive amino acids common to HLA-B27 and *Klebsiella pneumoniae* nitrogenase was identified. However, there was no reported association between *Klebsiella* and reactive arthritis, but the HLA-B27 residues 71 to 75 are identical to an epitope of the arthritogenic *S. flexneri* strains.

Immune Response and Immune Suppression Genes

Such genes, which have been demonstrated in mouse and guinea pig, form the basis for assumptions that at least some HLA-D/DR–associated diseases are due to altered or suppressed immune responses. In keeping with this concept, a number of diseases involving autoimmunity, or suspected to have autoimmune-components, have shown a stronger association with HLA-D/DR. Juvenile onset IDDM, Graves' disease, idiopathic Addison's disease, myasthenia gravis, dermatitis herpetiformis, celiac disease, chronic active hepatitis (lupoid type), Sjögren's syndrome, and systemic lupus erythematosus all have a significant association with Dw3 and DR3 in Caucasians. This type of association also seems relevant in multiple sclerosis and tuberculoid leprosy (DR2-associated). Cytotoxic antibodies to β-cells in the serum of patients with IDDM have been demonstrated, though these antibodies were also found in the serum of 25 per cent of first-degree relatives. Analysis of the gene sequences of MHC Class II molecules from patients with insulin-dependent diabetes mellitus suggested the inheritance of particular HLA alleles determining resistance to that disease. It is believed that the presence or absence of aspartic acid at position 57 of the HLA-DQ β-chain is a marker of the HLA-DQ β-allele associated with IDDM (Bell, 1989).

The most accepted hypothesis has been that autoimmune diseases associated with HLA Class II molecules involve an immune response of abnormal self recognized by T helper cells. The mouse model of myasthenia gravis (MG) is a good example. The immune response to the acetylcholine receptor (AchR) is under IR (immune response) genetic control. H-2^b mice respond to torpedo eel AchR and develop MG. The bm-12 mutant mouse, which carries a small mutation of the Class II allele I-A^b, neither responds to AchR nor develops the disease (Christadoss, 1985).

Receptor

It has been hypothesized that HLA antigens serve as receptors for pathogens. For instance, the Duffy red cell antigen acts as a receptor for malarial parasites and Duffy-negative individuals show correspondingly high resistance to malaria (Miller, 1975). In this case, susceptibility should be dominant. The HLA-A and HLA-B antigens have likewise been identified as receptors for Semliki Forest virus (Helenius, 1978), as well as other viruses.

Accidental

Some diseases show association for defective or absent proteins also coded for in the HLA region. Idiopathic hemochromatosis shows an association with HLA-A3. Partial biochemical expression is seen in heterozygous carriers, though progressive accumulation of iron in the liver occurs only in homozygotes (Amos, 1977; Cartwright, 1979). In a large collaborative study of hemochromatosis, a considerable excess of diseased sibs HLA identical with the proband supported a recessive mode of inheritance of a single gene.

HLA-Linked Genes

Defects in HLA-linked genes related to the complement system might be responsible for some HLA-related disease susceptibilities. Genetic linkage disequilibrium has been used to study chromosome segments as markers for diseases. For example, the association of C2 deficiency with the extended haplotype HLA-A25, HLA-B18, HLA-DR2, BFS, C4A4B2 (Alper, 1984).

HLA as a Marker for Abnormal Differentiation Antigens

Studies of H-2 have revealed a closely linked region which controls differentiation in the mouse (DeWolf, 1979). It is conceivable that certain HLA associations arise by linkage disequilibrium with abnormal alleles of human differentiation genes. For instance, human testicular teratocarcinoma is associated with Dw7.

Inappropriate Expression of HLA Class II Molecules

It has been suggested that HLA Class II molecules can be expressed on the target organ of an autoimmune disease—e.g., in thyroid epithelium. A virus infection could trigger a local immune response with T cell release of γ-interferon, which induces expression of Class II gene products. These HLA Class II expressing cells may initiate a continuous cycle of autoreactivity. T cells from thyroid tissue of a patient with Graves' disease can recognize influenza virus presented by thyrocytes after HLA-DR expression by γ-interferon (Bottazzo, 1983).

Shared Epitope of Class II MHC Gene Products

The striking correlation found between T cell recognition and the sharing of the HLA-DRB1 gene sequences (amino acids 67 to 74) among patients with rheumatoid arthritis suggested that the DR β-chain third variable region could be a restriction site for the putative causative agent in rheumatoid arthritis. The sequence is found in HLA-DR1; DR4,Dw14 and DR4,Dw15 DRB1 genes, supporting the concept that shared epitopes of different alleles may be involved in the pathogenesis of autoimmune diseases (Bell, 1989).

Involvement of Class III Genes in Disease

The involvement of deficiencies of C2 and C4 genes and the polymorphisms of C4 in disease has been reviewed previously. We mentioned that homozygous C2 deficiency and C4 deficiency are associated with a disease similar to systemic lupus erythematosus. The relationship between heterozygous C2 and C4 deficiency and the C4 null alleles with lupus has been suggested. However, linkage disequilibrium between polymorphisms of Class III genes and HLA-B and HLA-DR is so remarkable that any genes in an extended haplotype carrying a null allele may be relevant to genetic susceptibility. For example, if HLA-DR3 is associated with SLE, the *C4.4*Q0* that is in linkage disequilibrium will be found also associated.

It appears that more than one of the mechanisms just described may play a role in the pathogenesis of different diseases. For example, diseases with high relative risk for alleles of the HLA-B locus may be explained either by the altered self-mimicry or by the receptor hypothesis, whereas other diseases may involve HLA-linked genes that are directly or indirectly involved in regulation of the immune response. The finding that the 21-hydroxylase gene of the steroid-hormone pathway and the TNF genes are located within HLA also implies that impairment of some non-immunologic regulatory functions might produce HLA-associated disease.

SUMMARY

The major histocompatibility complex of man comprises many alleles at many loci. This polymorphism plays a major role in tissue transplantation, immune responses, and probably a wide variety of diseases of unknown etiology, including autoimmune disorders. Although not discussed at length in this chapter, new methods for sequencing MHC genes have extended the complexity of polymorphism and opened the way to defining the molecular correlates of human immune responses and determinants of a variety of diseases of unknown etiology.

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